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THE MECHANISM OF EVOLUTION IN THE GENUS *CLARKIA*

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INTRODUCTION

Clarkia is a genus of Onagraceae which consists of about 30 annual species endemic to western North America except for one in South America. This paper is based upon a 6-year study of all of the North American species both in their natural habitats and in the experimental garden. Its purpose is to examine the morphological, cytogenetical, and ecological relationships within the genus at levels from those within a population to those which characterize distinct species, and to suggest means by which the observed pattern of differentiation and divergence has evolved. It reports results obtained from morphological and cytogenetical studies of all known species of the genus and an extensive program of hybridization. The genetical work of Hiorth (1940, 1941, 1942, 1947 and others), and the cytological work of Håkansson (1940, 1941, 1942, 1943, 1946, 1949 and others) on certain species, has been taken into account and is correlated with my own evidence.

Most of the genetical and cytological studies in this group have appeared under the name *Godetia*. This genus is better considered congeneric with *Clarkia* for reasons which will appear elsewhere (Lewis and Lewis, unpub.). Names used by these authors are listed below with the synonyms used in this paper. This list does not include entities with the same specific name in both genera except where the names have been incorrectly applied.

Godetia amoena = *Clarkia rubicunda*
Godetia hispidula = *Clarkia arcuata*
Godetia nutans = *Clarkia gracilis*
Godetia parviflora = *Clarkia speciosa*
Godetia quadrivulnera = *Clarkia purpurea*
Godetia viminea = *Clarkia williamsonii*
Godetia whitneyi = *Clarkia amoena*
Clarkia elegans = *Clarkia unguiculata*

ECOLOGY AND BREEDING HABIT

The plant communities with which a given genus is associated can be taken as an index of its ecological diversity. Judged on this ground, the requirements of *Clarkia* are relatively uniform. Most species occur within the oak woodland, although some extend also into adjacent communities and some are characteristically associated with the lower margins of the montane forest, the grassland, and the chaparral which are adjacent to the woodland. Two species occur only on sea bluffs. However, even though the habitats of most species are similar, no two, even those associated exclusively with the woodland, have identical ranges.

Their ranges may overlap extensively and several species, often very closely related, may be found in the same area. As many as 7 species occur sympatrically in the oak woodland of the South Coast Ranges of California. The natural distribution of most of the species and their relation to each other has not been appreciably modified by man, even though some populations have undoubtedly been altered in size by activities such as road construction.

Clarkia is colonial and is restricted to relatively limited sites like most annuals found in similar areas. Its colonies range in number from a few to innumerable individuals which may sometimes carpet an area of several square miles. Perhaps the largest colonies are those of *C. cylindrica* in the grassland at the southern margin of the San Joaquin valley where their bright patches of color are visible many miles. Such large colonies are exceptional. The usual colony numbers several hundred or a few thousand individuals and covers several, perhaps 10, square meters. One may even occasionally find a solitary plant.

Colonies generally recur each year in the same sites and do not as a rule change appreciably in extent from year to year, in spite of extreme conditions of weather and contrary to the habit of many other annuals (*e.g., Layia,* Clausen, 1951). A known population has almost always been present when revisited in the appropriate season and in nearly all cases the density of individuals has been more or less the same. We have also observed that colonies may persist with very little apparent change for at least 20 years, even near the limits of distribution. For example, I have long known of two colonies of *C. dudleyana* near Redlands, California, which are marginal to the area of distribution. Even so, they have not obviously increased nor decreased in extent during this period, nor have I found other populations of this species in the vicinity, even though other sites nearby would appear to be suitable for them. Another indication that *Clarkia* populations show relatively little change in numbers at a particular locality from year to year has been the high degree of success we have had in locating populations when reasonably precise localities have been cited on herbarium sheets. This has certainly not been our experience when looking for *Delphinium, Eschscholtzia,* and species of other genera which have fluctuated tremendously during this period (Epling and Lewis, 1952). An exception to the constancy of *Clarkia* populations has been found in several colonies of *C. xantiana* near the margin of its range in the upper Kern River Canyon, California. Colonies which for three years had numbered several hundred individuals were much smaller in the spring of 1952. At one site only 25 individuals could be found.

Although established colonies usually show little change during the few years when one may observe them, there is evidence of appreciable movement of these species in relatively recent geologic time. This evidence will be discussed elsewhere. Marginal colonies doubtless disappear and, what may be of particular importance to the evolution of the group, new colonies obviously do become established. These new colonies are probably derived in many cases from a single seed or at least from very few.

The flowers of *Clarkia* are bisexual. The stamens are more or less simultaneous in development and those of a given flower ordinarily shed their pollen during 2 or 3 days. The stigma is normally closed at this time and does not become receptive until after the pollen is shed. It then expands and exposes a relatively large hairy glandular surface. The floral structure, therefore, favors outcrossing, particularly since the stamens are usually shorter than the style.

The flowers are visited extensively by various species of bees which are doubtless the principal pollinators. Fertilization occurs about 38 to 40 hours after pollination in the two species that have been measured (Johansen, 1927). Soon after

fertilization the flowers wither and abscise at the apex of the ovary. If fertilization is prevented the flowers may remain fresh for more than a week. The behavior of the flower can be used, therefore, as an index as to whether or not fertilization has occurred.

Outcrossing is promoted in most species by the relative length of the anthers and stigma and by different times of maturation. However, all *Clarkia* species are self-compatible and appreciable self-pollination is to be expected on most plants which have numerous flowers because bees usually visit several flowers of one plant before visiting another. Isolated plants with several flowers, therefore, normally produce at least some seeds by self-pollination, either by the action of insects, or by shedding pollen from the upper flowers onto the exposed stigmas of the lower ones. The pollen tends to hang together in strings and unless it is removed by insects, dangles from the anthers in fine threads which sooner or later are likely to blow across the prominent stigmas of the lower flowers. The pollen remains viable for at least a week in the laboratory.

This is the norm, from which a number of exceptions are found. Some entities, either species or races, are almost exclusively selfed. One race of *C. epilobioides*, for example, is even cleistogamous. It has been grown for several years in the garden and greenhouse at Los Angeles and for one year in London, England, and under 17 different conditions of temperature and light in the Earhart Laboratories at the California Institute of Technology. Although it has produced abundant seed in the garden and under several of the laboratory conditions, not a single flower has opened. Flowers of other races of this and of other species may open but they do so merely as a gesture, for pollination has already been accomplished. In such cases some or all of the stamens adhere to the stigma and deposit their pollen directly on it. In other species only the earliest flowers may open, the later flowers being completely cleistogamous. Outcrossing is rare in these cases but may occasionally occur. Furthermore, certain combinations of heat and drought may produce male-sterile flowers which are still capable of setting seed. Male sterility may therefore occur within parts of a population even though individuals in a slightly more favorable site are not affected. Still another type of self-pollination occurs, notably in certain races of *C. purpurea*. The style in these races is short so that the stamens are held against the stigma in the unopened flower, but the pollen is not shed until after the flower opens, unlike the self-pollinating flowers referred to above. When the pollen is shed the stigma has become receptive, but meanwhile the anthers have spread away from the stigma and are not in contact with it during the day. But the petals of these races close at night and thus bring the stamens into contact with the receptive stigma in a purely mechanical way. Therefore, if cross-pollination does not occur during the first day that a flower is open, self-pollination is likely to occur during the evening.

The number of seeds per capsule varies with the species and with the vigor of the plant. Furthermore, the earliest capsules of a plant have more seeds than the later ones. However, an average of about 50 seeds per capsule would perhaps be generally representative. A conservative estimate of the number of flowers produced per square meter in an ordinary population would be 1000 and an average colony would perhaps cover 10 square meters. An ordinary population might, therefore, be expected to yield at least a half million seeds per season. The capsules shed their seeds gradually, usually over a period of several weeks or even of months. The seeds are small, seldom as large as a millimeter in diameter, and have no apparent device to aid in dissemination. It seems certain, therefore, that the vast majority of the seeds produced are deposited within inches of the female parent. Some are

used as food by birds and doubtless by other animals as well, but the degree to which they are dispersed by these agents can only be guessed. There can be no question that some are carried widely on occasion, but we have no present measure of these distances nor the frequency with which wide dispersal occurs.

Seeds of *Clarkia* may remain viable for at least 5 years in the laboratory when stored under dry conditions at room temperature. We do not yet know, however, the extent to which seeds may remain viable on the ground from one year to the next. Many annuals, particularly those of arid regions, are known to persist for a number of years by virtue of seed storage. But the seeds of *Clarkia* have no dormant period and germinate at any time when conditions of moisture and temperature are favorable. Under appropriate conditions, germination is rapid and the percentage of germination is high. Large storage of seeds from year to year, therefore, does not seem probable because conditions for germination are almost certainly met each year throughout most of the range of the genus.

The density of individuals within a colony varies somewhat from species to species and from site to site. However, in most cases the number of seeds that germinate greatly exceed the number of plants that reach maturity. For example, Mr. Frank Vasek has found as many as 3900 seedlings of *C. deflexa* per square foot in the Santa Monica Mountains in late autumn although the maximum number of this species expected to mature in the same area would not exceed 50 per square foot.

The breeding habit and population structure of *Clarkia* plays an important role in the differentiation of colonies and hence eventually of species, as will be discussed below. Outcrossing will provide new gene combinations. At the same time, the prevalence of at least some degree of self-pollination fosters the expression of recessive mutants and gene combinations. Adapted traits, once they have become expressed, may rapidly become typical of a colony. However, morphological differentiation between colonies and eventually between species of *Clarkia* need not be attributed necessarily to adaptive changes but may to an appreciable degree be the result of random loss and fixation of particular alleles or gene combinations in small populations as indicated by Wright (1931, 1948). Such chance differentiation is probably most active during the initial stages of a newly established colony.

Variation within and between Diploid Species

Most clarkias resemble each other closely in many characters and form a closely knit and natural genus. Nevertheless, detailed studies have disclosed a great deal of variation between individuals of the same colony, between colonies, between geographical races comprised of many colonies, and between species. A trait which is sporadic in some populations may characterize others or may even be typical of some species. Therefore, differentiation within and between diploid species generally involves differences in the degree to which a trait is expressed.

Variation within colonies: A colony of *Clarkia* usually includes morphological, physiological, and chromosomal variants as well as differences in degree of fertility. Some variants appear with sufficient frequency to indicate that the genetic factors determining them are an established part of the population. On the other hand, others appear to be ephemeral and limited to sporadic individuals. The incorporation of some of the latter into the gene pool of the group seems improbable or at least rare, although under some circumstances they might have become frequent or dominant within the population.

An example of an ineffective variant is that of a tetraploid anther in an otherwise diploid flower of *C. unguiculata* (Lewis, 1951). Similar abnormalities doubtless recur in this and other species, but no

naturally occurring autoploids are known in the genus. An example of an ephemeral variant which might have persisted under some circumstances is found in a color variant of *C. cylindrica*. This species is normally outcrossed. Its petals are typically lavender with a dark red base. Two white-flowered plants were found close together in one large colony and may well have come from the seeds of a single capsule. The plants were small and had but two flowers each. At the time they were found each had one open flower and was shedding pollen that appeared to be good. The plants were tagged and were revisited later to obtain seeds. Neither had set seeds although all other plants observed in the same area had set full capsules. Seeds were collected from adjacent plants and a large population was grown from them, of which all were colored. Many of these plants were then self-pollinated but no white-flowered plants appeared in their progenies. The original population has been visited subsequently and no white-flowered plants have been found. Seemingly, therefore, the factor producing this character did not become a part of the gene pool of the population, perhaps because it was a sterile dominant. On the other hand, it may have been eliminated simply because the white flowers were not visited by pollinators. Discriminate pollination by insects is a well-known phenomenon (Grant, 1949; Mather, 1947). Furthermore, species of *Clarkia* which appear to be very similar may be differentially pollinated as we know from observation and progeny tests. Had an insect which preferred white flowers visited this colony of *Clarkia,* a white-flowered race might have become established. White flowers, although not necessarily determined by the same factors, occur sporadically in many species while mixed colonies of varying proportions of white and colored flowers occur in *C. unguiculata*. An entirely white-flowered colony is known in *C. xantiana* while one species, *C. epilobioides,* is characterized by white flowers.

The genetic variability of a natural population of *Clarkia* studied in its natural habitat is indicated in some measure by differences in color or color pattern of the petals, pubescence, and to some extent the conformation of the leaves, petals, and other parts. These differences remain largely unchanged when progenies are grown under uniform conditions. On the other hand, a very large part of the variation observed in natural populations of *Clarkia* is environmentally induced. Extreme modifications in size have been observed in all of the species. For example, a population of *C. amoena* may contain numerous large vigorous plants with many leaves and branches and with each of these individuals bearing dozens of flowers as much as 80 mm. in diameter. At the same time many depauperate plants with only two or three leaves and a single very small flower, not more than 5 mm. in diameter, can be found in the same population. The modification of the vegetative organs and accessory parts of the flower does not extend to the anthers and stigma to the same degree. The latter may appear to be oversized in depauperate individuals because of the relative reduction of the other flower parts. Between the extremes one finds an array of intermediates. These are demonstrable modifications that mostly disappear under uniform cultural conditions. Modification by the environment can obscure or even completely obliterate the morphological distinction and genetic discontinuity between some demonstrably valid species of annuals (*e.g., Eschscholtzia,* Lewis and Snow, 1951) but this is seldom if ever true in *Clarkia* when one can observe an entire population and not selected individuals.

Conspicuous differences in flowering time, rates of growth and other physiological responses have also been observed in natural populations of *Clarkia* but it is difficult to translate these differences into terms of genetic variability, even when progenies are grown under uniform or controlled conditions. For example, Mr.

W. R. Ernst observed a population of *C. williamsonii* in full flower in Yosemite Valley in early July. He returned a month later when the seeds were mature and found a new and distinct group that had just reached flowering in addition to the plants with ripe capsules. A month later he gathered seeds from the second crop. We have germinated seeds and grown plants from both collections under a variety of controlled conditions in the Earhart Laboratories and have found no differential response between them in any respect. The difference in time of flowering in nature may therefore have been the result of two distinct times of germination in that particular season. This may not have been due to a difference in the physiological adaptation of the two groups but perhaps merely to the position of the seeds in the soil with respect to such factors as temperature and the incidence of rainfall. However, the possibility has not been eliminated that the two collections might show a differential response to combinations of environmental factors that have not been tested.

Differences in physiological response can be shown within many colonies tested under uniform conditions. Only brief reference can be made to them because analysis has only begun. It is apparent, however, that the degree of physiological variability cannot be ascertained under any single set of experimental conditions. For example, random seed lots from a population of *C. williamsonii* were grown under a variety of controlled environments in the Earhart Laboratories. Under conditions of temperature and light which produced the best growth, relatively little variation in response was observed. Under more extreme conditions, however, conspicuous differences became evident (Lewis and Vasek, unpub.). However, a differential response by different genotypes within a population under marginal conditions may be of particular evolutionary importance to the group (Epling and Lewis, 1952).

Differences in fertility as measured by seed set and production of good pollen are apparent between individuals within natural populations of *Clarkia* and within progenies grown under uniform cultural conditions from seeds of these populations. Relatively few controlled intrapopulational crosses have been made and scored from which the degree of variation in seed set between different pairs of individuals can be demonstrated. However, we have found that certain individuals of *C. dudleyana* grown from seed from a natural population in Mariposa County, California, set no seeds when crossed reciprocally, although they were able to set full complements of seeds when crossed to certain other members of the same population. They also produced full capsules when open pollinated in the garden. Data on the production of visibly good pollen are also available for a few populations. Hiorth (1942a), for example, found a range from 96 to 22 per cent of good pollen within a progeny of *C. amoena* grown from seeds from a natural population. I have found a similar range in *C. unguiculata*. The greatest variation in apparent fertility is often found in populations with the greatest degree of variation in their chromosomal complements. The two phenomena are doubtless directly correlated for the most part.

Chromosomal differences within populations are known in several species of *Clarkia*, particularly *C. amoena* (Håkansson, 1942; Hiorth, 1942a) and *C. unguiculata* (Lewis, 1951). These differences involve both chromosomal rearrangements and chromosome numbers, but not polyploidy. Differences in chromosome arrangement have probably set the stage for many and perhaps all of the changes in chromosome number. Populations of some species will probably be shown eventually to vary in chromosome size.

The principal chromosomal rearrangements detected as heterozygotes within populations of *Clarkia* are reciprocal translocations which apparently involve

major portions of the chromosome arms. These translocation heterozygotes form rings or chains of chromosomes at meiosis which show a regular alternate segregation. The gametes which are eventually formed contain, therefore, complete genomes without duplication or deficiency. Consequently no measurable reduction in fertility is necessarily exhibited by these heterozygotes. In a great many respects they are not unlike those well known in *Oenothera* except that no ring involving a complete set of chromosomes is known, although in *C. amoena* 12 of the 14 chromosomes may be involved in some individuals (Håkansson, 1942). Furthermore, balanced lethal systems are not known in natural populations of *Clarkia*.

Reciprocal translocations as well as other structural rearrangements provide a mechanism whereby a particular gene combination may persist free from effective recombination (Darlington, 1936). At the same time genetical divergence may accumulate within these segments. A population may thereby become differentiated into a number of discrete groups of genotypes which may be very differently adapted as heterozygotes and homozygotes. If one of the homozygotes is better adapted within the area occupied by a particular colony it will eventually become fixed and characteristic of that colony. But if the heterozygote is superior to both of the homozygous combinations, as has been demonstrated for certain structural heterozygotes in *Drosophila pseudoobscura*, the frequency of the arrangements involved will remain variable within the population and fluctuate about a norm determined by the relative adaptedness of the combinations concerned (for review see Dobzhansky, 1951, Chap. V). Heterozygotes involving apparently the same reciprocal translocations are found in a number of different natural populations of some species of *Clarkia*. The occurrence of these heterozygotes is probably best explained on the basis of their superior adaptedness.

A very high frequency of translocation heterozygotes is found in some populations. This can perhaps be explained on the basis of superiority of the heterozygotes or may indicate recent and extensive hybridization between populations that have previously become differentiated in their chromosomal arrangements. Some of the natural heterozygotes, however, certainly indicate the former explanation. For example, heterozygotes consisting of one genome of 7 chromosomes and one of 6 are known in several natural populations of *C. amoena* (Håkansson, 1942). The genomes with 6 chromosomes have doubtless resulted from translocation and subsequent loss of a centromere region. These genomes are lethal as homozygotes or produce sterile dwarfs (Hiorth, 1948b). They certainly offer no competition to the heterozygotes. But it is difficult indeed to account for the presence of the genome of 6 in natural populations unless one assumes that the heterozygote, in spite of an apparent but perhaps not effective reduction in fertility, is also better adapted than individuals in the same population with two genomes of 7.

Translocation heterozygotes associated with a marked reduction in fertility, as a result of genetically unbalanced segregation of chromosomes or to crossing-over in the region of the chromosome arm proximal to the translocation, are generally not found in natural populations. This does not mean, of course, that they do not occur from time to time, but rather that they are almost inevitably lost or reach fixation. Such rearrangements will first occur in heterozygous condition and since reduction in fertility is involved they are for the most part doubtless eliminated. But populations of *Clarkia* may differ in rearrangements which as heterozygotes are associated with marked reduction in fertility, indicating that on some occasions they do become fixed and characteristic of populations.

Not all chromosomal changes are the result of reciprocal translocation. Some are undoubtedly due to inversion. The

latter have not as yet been identified in any natural population of *Clarkia*. However, inversions involving the centromere have been postulated (Lewis, 1951) as a reason for the failure of chiasma formation that has been observed in certain chromosomes of several species. The reason for the apparent absence or infrequent occurrence of inversion heterozygotes is not clear and may merely reflect too few observations. However, inversions of a size and position to permit 1 or 2 per cent crossing over, as those observed in natural populations of *Delphinium* (Lewis *et al.*, 1951), would certainly have been noticed were they at all frequent.

Chromosome numbers may vary within particular populations of several species (Håkansson, 1942, 1945, 1949; Lewis, 1951), due in most cases to the presence of from 1 to 6 chromosomes in addition to the normal basic set in some, but not all, of the individuals. To judge from their probable origin (Lewis, 1951) they are duplicates of chromosomes that may form part of the basic complement and are therefore presumably genetically active. However, these extra chromosomes are not associated with any morphological or physiological traits that have yet been detected. The fact that no differences are ordinarily associated with these chromosomes under the conditions in which they have been observed does not mean that under some, perhaps exceptional, conditions they may not produce adaptive traits.

The normal number of chromosomes may also be decreased. For example, *C. amoena* usually has two sets of 7 chromosomes each but in 5 populations individuals have been found with one genome of 7 and one of 6 (Håkansson, 1942; Hiorth, 1942a). The genomes with 6 are genetically different in each of the 5 populations but behave similarly in that all of them occur only as heterozygotes (Håkansson, 1946a). Upon selfing the heterozygotes a few sterile dwarf "nullisomics" with $2n = 12$ were obtained in some cases but none were produced in others (Hiorth, 1948b). However, by crossing plants with 13 chromosomes from different localities, one apparently normal and fertile progeny with $2n = 12$ was produced (Hiorth, 1948b; Håkansson, 1946a). Similar individuals may eventually be found in nature.

The genomes with the reduced number have apparently resulted from translocation of most of the active genetic materials from one chromosome to the arms of others followed by the loss of the remainder of the chromosome, together perhaps with other small translocated portions. These deficiencies apparently prevent normal development as homozygotes but are compensated for in the fertile 12 chromosome hybrids mentioned above (Håkansson, 1946a). This suggests that the genomes with a reduced number have arisen independently on more than one occasion or have subsequently become modified through additional rearrangement. In either case it emphasizes the fact that structural rearrangements frequently become established in *Clarkia* populations.

Differences between colonies: The frequency with which certain traits are expressed may differ between colonies or parts of colonies. These differences are sometimes abrupt or discontinuous and sometimes they tend to be clinal.

Sharply defined differences in such characters as flower color, petal markings, pubescence of the ovary and similar traits can often be observed between adjacent colonies. For example, *C. xantiana* characteristically has bright lavender flowers. However, a population is known in the upper Kern River Canyon that consists of several hundred individuals with white flowers. A population of similar size which is uniformly of the typical color occurs only 100 feet away. The two are separated by a rocky area which is unsuitable for *Clarkia*. No discernible change in flower color has occurred in either for three years. Two explanations are possible. The flower color of each

may be associated with factors which make it particularly adapted to the site where it is found; or the white color may have resulted from chance fixation of a particular allele or gene combination. The second explanation seems more likely, for a white-flowered individual has been found in an otherwise typical population a half mile up the canyon from the white population. Therefore, the white colony may have been derived from a single seedling which happened to be homozygous for white color, or, if heterozygous, this trait may have become established in the early stages of the colony.

Similar differences which have probably had their origin in the same way may also characterize groups of colonies. *Clarkia biloba,* for example, ranges in the Sierra Nevada foothills from Butte County south to the Merced River in Mariposa County. The petals are pale pink or lavender as far south as the north bank of the Merced River at Bagby. Directly across the river, and for 20 miles along the south side of its canyon, the populations are an intense pink, approaching magenta.

Adjacent populations of *Clarkia* often differ in quantitative characters such as size and conformation of the leaves, petals, and other organs. These differences may not be obvious in natural populations because of environmental modifications but may be readily observed when progenies derived from different natural populations are grown under uniform cultural conditions. The differences between adjacent populations may be conspicuous or barely perceptible but they seldom show discontinuous variation as indicated above for flower color. This is doubtless due to the fact that quantitative differences are determined in large measure by polygenic systems.

Clines in the length-width ratio and degree of lobing of the petal have been found by analyzing material of *C. biloba* collected in nature (Roberts, 1952). A genetic basis for these clines has been confirmed by growing progenies under uniform garden conditions. Similar clines may be found in other species when they are intensively studied.

Physiological responses such as rates of growth, time of flowering, and susceptibility to disease vary among progenies of a species grown under uniform conditions. The differences are most conspicuous when populations from the most extreme habitats are compared. For example, seedlings of *C. unguiculata* from near sea level grow much more rapidly and flower much earlier in the greenhouse at Los Angeles than do those from 4000 feet in the Sierra Nevada. On the other hand, the variation between adjacent populations of the same species, in the few cases where data are available, overlaps to such a degree that differences can only be detected by a comparison of mean response. In this respect *C. unguiculata* is similar to *Achillea* (Clausen, Keck and Hiesey, 1948).

Ecotypes comparable to those described in *Potentilla* (Clausen, Keck and Hiesey, 1940) are occasionally found in *Clarkia.* For example, both *C. amoena* and *C. rubicunda* have broad-leaved, thick-stemmed coastal races which grow on sea bluffs and which retain their morphological distinctions when grown in the experimental garden. These races are apparently interfertile and form a zone of intermediates with the more graceful inland populations of their respective species. On the other hand, populations or groups of populations which are morphologically similar may be differentiated chromosomally and may possess strong barriers to gene exchange. In this respect *Clarkia* is very similar to *Holocarpha,* a genus of annuals in the family Compositae (Clausen, 1951).

Chromosomal differentiation between populations may involve different frequencies of particular chromosome arrangements or number of supernumerary chromosomes. On the other hand, the differences may be discontinuous. Differences in frequency of particular chromosome arrangements in *C. amoena* have

been shown by Håkansson (1942) and Hiorth (1942a) by crossing plants derived from seeds from natural populations with those of known chromosome arrangement. A difference in the frequency of supernumerary chromosomes is exemplified by colonies of *C. unguiculata* along an 8-mile transect in San Luis Obispo County, California, where the percentage of individuals with supernumerary chromosomes may vary conspicuously (from 0 to over 70 per cent in colonies for which 5 or more individuals were examined) (Lewis, 1951). Discontinuous differences in chromosome arrangement are indicated by the production of progenies consisting of structural heterozygotes from crosses between populations that are apparently structurally homozygous. This has been shown in *C. amoena* (Håkansson, 1942; Hiorth, 1942a) and in *C. unguiculata* (Mooring, unpub.). Translocations are primarily responsible for the structural heterozygotes although one interpopulational hybrid of *C. amoena* was found to be an inversion heterozygote (Håkansson, 1942). Consistent differences in chromosome numbers between populations as have been found in *Holocarpha* (Clausen, 1951) are not known in *Clarkia* although it would not be surprising if they were eventually shown to occur.

Barriers to gene exchange between populations of the same morphological species may prove to be a phenomenon of wide occurrence in *Clarkia*. Hybrids between populations are often much less fertile than comparable ones within a population and some are difficult to obtain. For example, in *C. deflexa,* a well-defined species of southern California, morphological differences are discernible from population to population but they are of no greater magnitude than those frequently found within species of annuals. Approximately 200 interpopulational crosses involving 31 combinations of 11 different populations have been made. The results are summarized in figure 1. Some of the crosses were fully fertile, others produced F_1 hybrids

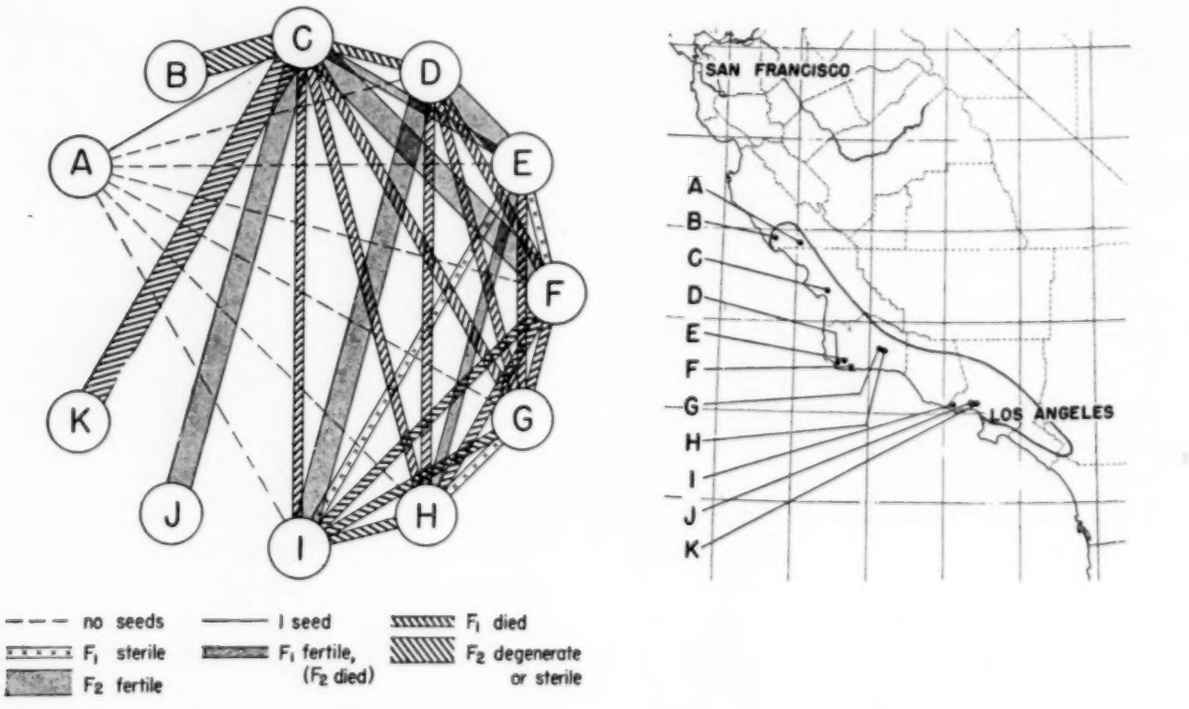

FIG. 1. Fertility relationship of populations of *C. deflexa* having the geographical distribution indicated. Progenies indicated as having died without issue were in some cases victims of disease; in others they were not pollinated.

that were apparently sterile or in which the F_2 was largely degenerate or sterile. Furthermore, when individuals from one population (A) were crossed to 7 other populations a total of more than 50 times only one viable seed was obtained. The F_1 hybrid that grew from this seed was highly fertile. However, the F_2 segregated into both partly fertile and sterile individuals. So far as present data are concerned, no direct correlation between geographic distribution and fertility is apparent. However, a two-dimensional representation of geographical relationships of this sort may be misleading. For example, the intersterile populations G and H are separated by a distance of no more than 5 miles but are separated by at least 2000 feet in elevation and are on opposite sides of a ridge.

Restriction of gene exchange between populations similar to that indicated for *C. deflexa* is known in several other species. Crosses between populations of *C. dudleyana* from southern California and the Sierra Nevada produce vigorous but sterile hybrids. Fertility is reduced to about half in hybrids between certain populations of *C. biloba* (Roberts, 1952). In both cases meiosis in the hybrids is apparently normal. The cause of the reduction in fertility may be either genic or cryptic structural hybridity. In view of the frequent occurrence of structural hybridity within many populations of *Clarkia* one may expect it to be a major factor involved in the observed reductions in fertility. However, the cytological observations necessary to demonstrate the role of structural rearrangements in this respect have only begun. At the same time, it is apparent that the barriers in at least some cases are genic as, for example, the inability of one population of *C. deflexa* to cross with others. Another example is found in *C. amoena*. Progenies derived from interpopulational crosses in this species may include various proportions of seedlings that die in the cotyledon stage or show arrested development. Hiorth (1946, 1948c) has found these to be due to a complex of complementary factors. These factors may have no detectable effect within a populaton but may be lethal when combined with genotypes of other populations. These remind one of the well-known interspecific complementary lethal gene found in *Crepis* (Hollingshead, 1930).

The barriers to gene exchange between different populations of the same morphological species in *Clarkia* are apparently similar both in kind and degree to those generally found between species.

Relationship between species: Recognizable species of *Clarkia* may differ in a single detectable morphological character, or they may differ in characters which would justify generic segregation were they the only species to be considered. For example, *C. biloba* and *C. lingulata,* which are intersterile and have genomes of 8 and 9 chromosomes respectively, are separated morphologically only by the shape of the petal. On the other hand, *C. amoena* and *C. breweri* differ not only in a complex of characters which might of themselves justify generic segregation, but the latter has 4 rather than 8 stamens and in this respect is not even characteristic of the family Onagraceae. However, other species represent morphological intermediates and thereby unite *Clarkia* into a natural group.

The taxonomic value of characters may vary greatly from entity to entity as is so often the case. Characters which are typical of some species may occur as sporadic variants in others. For example, a particular pattern of lobing of the petals characteristic only of *C. xantiana* has been found as an occasional variant in *C. amoena* (Hiorth, 1941), in which the petals are generally unlobed.

The morphological differences between two species may in some cases be less than the differences within these species. For example, populations of *C. biloba* from the northern part of its range are scarcely to be distinguished from some populations of *C. dudleyana,* a species which is sympatric with *C. biloba* only in

the southern part of the range of the latter. Furthermore, the two species differ in chromosome number and we have been unable to cross them. On the other hand, populations of *C. biloba* from opposite ends of its range differ in a number of characters including the color, size, proportions and degree of lobing of the petals, the size and conformation of the leaves as well as differences in gross habit. At the same time, the southern populations differ from *C. lingulata,* as indicated above, only in the shape of the petal.

Closely related species of *Clarkia* generally have similar ecological requirements. Nevertheless, they tend to replace one another geographically. For example, *C. amoena* occurs in the coastal ranges from Marin County, California, to British Columbia. Its closest relative, *C. rubicunda,* is found in the coastal ranges to the south of *C. amoena* but the two ranges overlap in southern Marin County. Furthermore, changes in chromosome number at the diploid level are correlated with differences in ecological preference (Lewis, in press). This would suggest that ecological differentiation has often if not always accompanied speciation in this genus.

The chromosome complements of different species may differ with respect to structural rearrangements, chromosome size, and chromosome number.

Differences in chromosome arrangement between species are common, to judge from the meiotic irregularities observed in interspecific hybrids. Univalents are the rule, with relatively few pairs or multivalents. The multivalents indicate that many of the rearrangements are translocations but inversions may also occur. This is indicated by the report of an inversion bridge in a hybrid of *C. bottae* and *C. deflexa* (Håkansson, 1943b). Meiotic irregularities may also be due to genic as well as chromosomal factors (Beadle, 1932, 1933). However, in *Clarkia* indirect evidence indicates that the basis of meiotic irregularities in interspecific hybrids is largely chromosomal. One-third of the species are alloploids and the hybrids between their inferred parents are highly irregular at meiosis. These irregularities are obviously due to chromosomal rearrangements for should they have been due to genic factors meiosis in the polyploids would be expected to be equally irregular, which is not the case.

Species of *Clarkia* with relatively large chromosomes and those with relatively small ones are about equally divided. The direction of change has probably been from large size to small. Hybrids between those with large and small chromosomes show that the size differences of each genome are retained in some hybrids (*C. bottae* × *C. deflexa,* Håkansson, 1943b; *C. biloba* × *C. deflexa,* and others, Lewis, unpub.) similar to the hybrid in *Crepis* reported by Tobgy (1943). The size of individual chromosomes in these cases seems to be intrinsically controlled by each chromosome. On the other hand a hybrid between *C. williamsonii* which has large chromosomes and *C. speciosa* which has small ones, had chromosomes all of one size class. This would indicate that the size of individual chromosomes in this case was determined by the genotype as a whole.

Differences in chromosome number between species of *Clarkia* represent deviations from an original basic number of 7 (Lewis, in press). A genome of 5 is characteristic of one species, *C. virgata,* and has probably resulted from translocations followed by the loss of two centromere regions. Genomes of 8 and 9 have apparently been derived from 7 by the addition of the equivalent of whole chromosomes.

One of the clearest examples of the addition of a whole chromosome is found in *C. lingulata.* This species is known from only two localities along the Merced River. It has 9 chromosomes and is very closely related to *C. biloba* which has 8. Most species differ so greatly in chromosomal rearrangements that it is virtually

impossible to determine the homologies between their chromosomes and thus ascertain the mechanism of change in chromosome number. However, pairing of the chromosomes in the hybrid between *C. biloba* and *C. lingulata* is regular and the 9th chromosome of *C. lingulata* normally enters into multivalent configuration (Roberts and Lewis, unpub.). This indicates that the additional chromosome is homologous at least in part to the normal genome of *C. biloba.* This increase in chromosome number may have come about either from hybridization of two species with similar genomes or from duplication of a chromosome within a species.

Hybrids between closely related species of *Clarkia* usually show mostly univalents at meiotic first metaphase and are consequently highly sterile. However, at least a few pollen grains appear to be viable and occasional functional egg cells are doubtless formed. Some may very well contain a complete genome of one species plus one chromosome from the other. A chance combination of two such gametes could result in a viable individual with an additional pair of chromosomes. Should this individual be adapted to the site in which it finds itself it might thrive and give rise to a new race. This race might prove to be incompatible with the parent from which most of the chromosomes were derived and might also differ morphologically. With this hypothesis in mind, attempts have been made to see whether *C. lingulata* might have had this origin by crossing *C. biloba* to *C. dudleyana* and *C. modesta,* which grow in the same general area. The only hybrid so far obtained has been one with *C. dudleyana* which formed no flowers whatsoever. The possibility that the additional chromosome of *C. lingulata* has been derived through hybridization cannot yet be ruled out, however, because the results of hybridization may differ greatly depending on the individuals involved. We have recently obtained a few seeds from a cross between *C. biloba* and *C. modesta* which may prove to be viable.

Duplication of a chromosome within *C. biloba* has also been investigated as a possible source of the additional chromosome in *C. lingulata.* Chromosomes that are probably duplicates of those that form part of a normal basic set occur as supernumerary chromosomes in several species (*C. gracilis,* Håkansson, 1945; *C. williamsonii,* Håkansson, 1949; *C. unguiculata, C. rhomboidea,* Lewis, 1951). Such supernumeraries are probably a by-product of meiosis of certain structural heterozygotes that are frequent in natural populations. Ways in which these and similar extra chromosomes might become a part of the normal basic set have been suggested (White, 1945; Hiorth, 1947b; Lewis, 1951). If the additional chromosome of *C. lingulata* did arise by duplication of a chromosome in *C. biloba,* one would expect to find in populations of the latter either supernumerary chromosomes or structural heterozygotes from which they could regularly be produced. Neither has yet been found, but samples have been small because of the difficulty of obtaining plants in nature with a bud in active meiosis.

Barriers to gene exchange between species of *Clarkia* are extremely well developed. Hybrids between the diploid species are frequently very difficult if not impossible to obtain. A total of 117 interspecific hybrid combinations have been attempted, often repeatedly, but only 19 of these have produced a viable F_1. These hybrids have all been sterile or nearly so when self-pollinated, backcrossed to either parent, or open pollinated in the experimental garden. Several reasons account for the failure to hybridize and the sterility of those hybrids that are produced. In some cases the pollen does not grow in the style, in others pollen growth is normal but fertilization apparently does not occur. Abscission of the flower may occur after interspecific pollination, indicating that fertilization has occurred, but only shrivelled

seeds may be formed. The sterility of those hybrids that have been produced is apparently due to extensive repatterning of the chromosomes in most cases but sterility genes may also be a factor in some hybrids.

Naturally occurring hybrids between diploid species have not yet been found. Nevertheless, such hybrids must occasionally be formed to account for the numerous alloploid species.

Discussion

Evolution at the diploid level in *Clarkia* has involved extensive chromosomal and probably genic changes which have led to the formation of barriers to gene exchange as well as morphological and ecological differentiation between populations and eventually between species.

Individuals within a population may vary from one another in morphology, physiology, chromosome arrangement, chromosome number, and in their capacity to exchange genes. At the same time, the variants found within some populations may characterize others. Furthermore, the differences that distinguish populations within a species are the same, except perhaps in degree, as those that differentiate species. Therefore, the mechanism of speciation can be accurately inferred from a study of the mechanisms involved in the differentiation of populations, except, of course, mechanisms of speciation such as alloploidy which result from hybridization between previously delimited species.

Morphological differences which distinguish populations or species may result from differences in adaptedness. The morphological expressions may themselves be adaptive or they may be determined by genotypes which are highly adapted in spite of, or at least independent of, morphology determined by the genes involved. On the other hand, morphological differentiation may be due to chance fixation of alleles as a result of random fluctuations in small populations. Random loss and fixation may be a common phenomenon in *Clarkia*, particularly at the time of establishment of new colonies from a meager beginning followed by close inbreeding.

Adaptive differentiation has doubtless played a more constructive role in the evolution of *Clarkia* than has random fixation. But the latter may be responsible for many of the trimmings. However, it is not always easy to distinguish between phenomena attributable to chance alone and those that are adaptive. Furthermore, an adapted trait under one set of conditions may not be adapted under another. For example, small flower size characterizes all habitually self-pollinating races of *Clarkia* in contrast to the related outcrossed races or species. The two traits are independently determined by polygenic systems, at least in the one species, *C. purpurea*, for which we have data. This suggests that large flowers are better adapted than small flowers in an outcrossing race while the converse is apparently true when self-pollination is the rule. Large flower size may be maintained in the former by a selective preference for large flowers by the insect pollinators while in the latter case it has been suggested by Dr. Verne Grant (personal communication) that the adaptive value of small flowers may lie in the rapidity with which they may be formed with greater economy of materials.

Adapted gene combinations arise by chance and those that are of particular significance in evolution are the ones that happen to be associated with mechanisms which transmit them to subsequent generations without fundamental change. This is not to say that recombination is unimportant to evolution but merely that any particularly adapted genotypes which may arise are of little significance if they are destroyed by recombination and cannot be transmitted to subsequent generations (Darlington, 1939).

Structural rearrangement of chromosomes is of prime importance as a mechanism which permits adapted gene combinations to persist immune from recom-

bination and for this reason is probably the most important single factor in the evolution of *Clarkia,* particularly with respect to species formation.

The frequency with which chromosomal mutations involving structural rearrangement occur is not known. However, the vast majority of rearrangements that do occur are doubtless eliminated by chance or because they do not happen to involve gene combinations adapted to the prevailing conditions. On the other hand, when a rearrangement is associated with a gene combination better adapted to the site in which it occurs, it may become established and eventually replace the original arrangement in the population. Hybrids between populations and particularly between species of *Clarkia* indicate that such replacements have recurred repeatedly in the differentiation of many populations and all species. The number of chromosomal arrangements that differentiate most species cannot be estimated, but the extremely low degree of homology between genomes indicated by the very low frequency of bivalents in nearly all interspecific hybrids suggests that the number may be very great. This is particularly true if one takes into account the numerous small rearrangements that may be present but undetected. For example, Stebbins (1950) has indicated that in most diploid species 30 to 50 rearrangements may be present without an apparent disturbance of meiosis.

Some rearrangements such as short inversions may not in themselves be associated with an effective reduction in fertility in structural heterozygotes while other rearrangements may result in a reduction of fertility as high as 50 per cent. However, even those that do not effectively reduce fertility may contribute in combination to a reduction in meiotic pairing in hybrids and this in turn may result in a reduction in fertility. A by-product of the accumulation of numerous structural rearrangements in different populations within a species, has, therefore, been the development of barriers to gene exchange, which may eventually result in speciation.

The breeding structure in *Clarkia* is one which facilitates the accumulation of structural rearrangements because of the prevalence of at least some degree of self-pollination in vigorous individuals of all species. Self-pollination affords an opportunity for expression as homozygotes of gene combinations which may not be particularly well adapted as heterozygotes. Should these homozygotes be better adapted they, and the chromosome arrangement with which they are associated, may become typical of the entire population.

Chromosome rearrangements may remain in a population for an indefinite period without becoming lost or typical. In *Clarkia* structural heterozygotes are frequently found in natural populations. It seems probable that many and perhaps most of these naturally occurring structural heterozygotes are associated with gene combinations that are better adapted than those of either homozygote in a manner similar to that which has been demonstrated in certain other organisms (*e.g., Drosophila pseudoobscura,* Dobzhansky, 1947; *Campanula,* Darlington and LaCour, 1950; *Paeonia,* Walters, 1952). As long as this relationship is maintained a mixture of chromosome arrangements will persist in the population unless an arrangement is lost by chance. However, should one of the genotypes become better adapted as a homozygote due, for example, to a change in the environment, the particular arrangement involved would then be expected eventually to become characteristic of the entire population.

Chromosomal arrangements that are able to enter into persisting adapted combinations usually do not effectively reduce fertility and therefore will not constitute of themselves sterility barriers between populations until they have accumulated to such an extent that normal meiosis is disrupted. At the same time,

since effective crossing-over is essentially eliminated in the rearranged segments of most structural heterozygotes, differently adapted genotypes that are effectively immune from recombination may be built up within a single population and may eventually become characteristic of different populations. Colonies that have become differentiated for several such blocks of genes may form hybrids which are fully fertile. However, as a result of recombination, few if any of the progeny may be sufficiently well adapted to survive and reproduce under natural conditions, or for that matter even in the experimental garden. This may perhaps be exemplified by several known interpopulational crosses in *Clarkia* in which the F_1 is apparently fertile but the F_2 is largely degenerate. The accumulation of structural rearrangements, therefore, may lead directly to the production of barriers to gene exchange between populations by way of a sterile hybrid or, where the rearrangements themselves do not effectively reduce fertility, an effective barrier may result from the accumulation of genetic differentiation in the rearranged segments of the chromosomes. In either case these barriers lead potentially to speciation.

Although the accumulation of structural rearrangements in the manner outlined above has undoubtedly played a major role in the formation of barriers to gene exchange and hence to speciation in *Clarkia,* it is not necessarily the only factor concerned. Complementary lethal factors are known within at least one species and similar factors may account for the failure to obtain many interspecific hybrids. However, one does not know in these cases whether genes preventing effective hybridization were first to form a barrier to gene exchange or whether they have since been added to other barriers that were already effective.

Interspecific hybridization may also play a role in the formation of new species by means other than polyploidy. This would be possible should a genotype of interspecific hybrid origin be particularly adapted to the site in which it found itself and at the same time was sufficiently fertile to leave progeny. Selection in subsequent generations might eventually produce a fertile entity with the parental chromosome number but differing in having a genotype that combined parts of both parental genomes. This new entity would probably be differently adapted and perhaps morphologically distinct from both of its parents. Speciation of this kind has probably occurred in a number of genera (*e.g., Delphinium,* Lewis and Epling, 1946 and unpub.) and may have been effective in *Clarkia* although it has not as yet been demonstrated. However, it has been demonstrated that the process is possible in this genus. An interchange between genomes of *C. amoena* and *C. deflexa* was produced in their sterile hybrid by Hiorth (1942b) using x-ray. Subsequently a fertile strain was established that was similar to *C. amoena* but differed in having at least one morphological character derived from *C. deflexa.* This race may also have been differently adapted from either of the parents.

Speciation in *Clarkia,* as outlined above, has probably resulted from a combination of a variety of factors. Of these perhaps the most important has been the differential accumulation in different populations of adapted gene combinations associated with particular chromosome rearrangements. A by-product of this accumulation of structural rearrangements has been the establishment of strong barriers to gene exchange.

Speciation in obligatorily outcrossing species is generally conceded to result from a gradual accumulation of small differences which will eventually prevent or greatly restrict gene exchange between the differentiated populations. In this slow process one may expect that factors which produce morphological and physiological differentiation as well as factors limiting genetic exchange will be more or less simultaneously accumulated. Therefore, in such groups the degree of mor-

phological differentiation will be a more or less reliable clue to specific differentiation.

But in *Clarkia,* as we have seen, a correlation is not necessarily found between morphological differentiation and the capacity to exchange genes. Morphologically similar populations may be intersterile. At the same time, populations may be conspicuously differentiated morphologically in the absence of apparent potential barriers other than distance. The former differentiation is essentially irreversible and may lead to species formation; the second may be a temporary phenomenon. These phenomena both suggest that differentiation in *Clarkia* is often and perhaps usually a rapid process. This may result primarily from a colonial habit, self-pollination, and perhaps selective pollinators.

Colonial habit may be a factor in rapid differentiation as a result of random fixation of particular alleles or chromosome arrangements. This may be due to the small size of some colonies but is of particular significance with respect to rapid differentiation in the initial stages of colony formation. However, of perhaps greater importance is the potentiality of self-pollination in the more successful individuals in all species. Any linked gene combination that is particularly well adapted in a given site as a homozygote will have an excellent chance to become expressed as a result of even a small amount of selfing and subsequently may rapidly become typical of the population. Furthermore, when such a gene combination is held immune from recombination by a structural rearrangement of the chromosomes that rearrangement will simultaneously become typical of the population. A rapid differentiation with respect to structural rearrangement, which is doubtless a major factor in establishing barriers to interpopulational gene exchange, need not be associated with conspicuous morphological differentiation.

Differentiation between populations that prevents or greatly restricts further gene exchange may not only arise within a few generations in *Clarkia* but may arise with great frequency. These barriers may be comparable to those generally found between species. This does not mean, however, that all or even most of the populations that become separated by strong barriers to gene exchange will eventually become species as we normally consider them. On the other hand, it seems almost certain that the vast majority of these genetically isolated groups are short lived and eventually become extinct. This seems probable because they must rely for their adaptiveness upon a limited genetic variability inasmuch as they no longer have the possibility of deriving additional variability from other populations. The success of the genus seems not to lie primarily in the gradual segregation of adaptive races but rather in the production of a multiplicity of locally adapted, genetically isolated, and often ephemeral segregates. The genus *Clarkia* as we see it today consists obviously of those segregates that have become successful species, some of which have apparently persisted for a considerable length of time. With these are a number of more recent genetically isolated populations, a few of which may develop into the successful species of the future.

The pattern of evolution indicated by *Clarkia* is very different from that which is apparently general in other organisms, particularly among higher animals and perennial plants. One excellent recent account of the general process of speciation in plants is that of Clausen (1951), an account which is based upon an exceptionally broad background of personal observation and extensive cooperative experimental studies with his colleagues. The first stage as presented by him is a morphological differentiation of local populations followed by differentiation of groups of these populations into ecological races. These ecological races may in some cases be equivalent to morphological subspecies. The final step in speciation is the evolution of interspecific barriers to

gene exchange. Even granting that some of these processes do not form definite "stages" but instead may over-lap or proceed simultaneously to varying degrees, the pattern still does not fit that indicated by *Clarkia*. However, many of the examples from the annual Madiinae, a tribe of Compositae, cited by Clausen (*l. c.*) are very similar to *Clarkia*.

The reasons for different patterns of evolution are several. For example, the difference in breeding habit between obligatory outcrossing and even a small amount of self-pollination will certainly affect the pattern. The same is true of the many factors which determine population structure. For example, species which are not broken into more or less discrete colonies will not be expected to show characteristics of differentiation that may be associated with the establishment of new colonies or with small disjunct colonies. The degree of over-lap between generations will also be a factor. Add to this the kinds and frequency of chromosomal and gene mutations plus the environments to which the genotypes are subjected and one will find ample basis for differences in patterns of evolution.

So many variables are involved in the pattern of evolution of any group of organisms that one may ask with justification whether each such group will have to be examined in detail before reasonably accurate and inclusive generalizations can be made concerning patterns of evolution. On the other hand, there is no reason to believe that these patterns are infinite in number. It seems reasonable to predict that the pattern indicated by *Clarkia* is essentially the same as that of other groups of annuals with comparable breeding habit, colonial structure and occupying areas of comparable ecological diversity.

Summary

The genus *Clarkia* is comprised of species of annuals that occur in disjunct colonies of various size. The flowers are characteristically insect pollinated and for the most part designed to promote outcrossing. However, all individuals are normally self-compatible and at least some degree of self-pollination is general.

Different species as well as populations within a species are characterized by the same kind of traits that may distinguish individuals within a population. Morphological, physiological, and chromosomal differences, including differences in chromosome arrangement and number, as well as differences in potential fertility are found to vary between individuals within many populations.

Furthermore, the differences which characterize species are reflected in the differences found between populations within a species. The latter may involve not only morphological, physiological, and chromosomal differences but also the capacity to exchange genes. A complete spectrum exists extending from those colonies that are highly interfertile to those in which the F_2 is partly or largely degenerate, to those in which the hybrids are but partly fertile or even sterile and finally to those that are unable to cross.

Morphological and perhaps chromosomal differentiation between populations may in many cases have resulted from chance loss and fixation of particular alleles or chromosome arrangements in small populations, particularly as a consequence of the meager beginning and subsequent inbreeding that of necessity occurs in establishing a new colony. However, adaptive changes have doubtlessly played a more fundamental role.

Speciation has been a by-product of the production of barriers to gene exchange in the course of adaptive and chance differentiation. The factors contributing to the production of these barriers are several. However, the most important single factor contributing to speciation in this group has probably been the differential accumulation of chromosomal rearrangements. This accumulation, either by chance or more frequently as a result of association with adapted gene combinations, will immediately or eventually lead to a reduction in fertility

between different populations. Furthermore, chromosomal rearrangements have been directly or indirectly involved in changes in chromosome number at the diploid level. At the same time, by reducing the structural homology between genomes, the accumulation of structural rearrangements has set the stage for successful alloploidy.

The occurrence of populations which are intersterile but morphologically similar, as well as adjacent populations that are morphologically distinct but apparently interfertile, suggests that differentiation which may lead to speciation in this genus may frequently occur rapidly. Most of the genetically isolated populations thus formed are probably short lived, but a few may become successful species. Gradual segregation of geographic races also occurs but the success of the genus seems not to lie so much in the gradual segregation of adaptive races as in the production of a multiplicity of locally adapted, genetically isolated and often ephemeral segregates.

It is suggested that patterns of evolution similar to that of *Clarkia* will be found in other comparable groups of annuals.

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COMPARATIVE EVOLUTION WITH SPECIAL REFERENCE TO PRIMITIVE MECHANISMS

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"Comparative Evolution is destined to become as important a branch of biology as Comparative Anatomy."—Huxley.

INTRODUCTION

The subject of "comparative evolution" (Huxley, 1942; White, 1945) has not received the attention which it deserves. Current views regarding the nature of evolutionary mechanisms are based mainly on the behavior of highly specialized organisms, themselves the products of hundreds of millions of years of evolutionary selection and fixation. These views presume that there has been little or no evolution of evolutionary mechanisms in about two billion years—a most unlikely presumption! It is our belief (1) that there has been an evolution of evolutionary mechanisms as well as of the products of such mechanisms, and (2) that evolutionary mechanisms, as well as their products, may be primitive or advanced, generalized or specialized, and may evolve in a divergent, parallel or convergent manner.

The evidences which bear upon the analysis of specialized evolution lie all about us and have been intensively studied in the last few decades. A host of workers has examined these evidences and the general consensus of opinion in regard to specialized evolution in animals is that it depends upon the occurrence of shifting gene frequencies in cross-fertilizing organisms, followed by the development of reproductive isolation, which leads to subspecific and thence to specific origins and ultimately to the evolution of all the higher categories. This Neo-Darwinian interpretation has been carefully developed and expounded in the recent books of Dobzhansky (1951), Mayr (1942) and Simpson (1944), who present, from the viewpoints of Genetics, Systematics and Paleontology, the theory that all evolution is subspeciational. But this is theory and not proven fact for no one has as yet been a witness to the production of a single animal species by such processes. Furthermore even the best-documented evolutionary or phylogenetic sequences are but fragmentary records of what is assumed to have happened on the theory of continuous gradation, and the hopes of actually witnessing the production of a new animal species are very remote if the estimates of Haldane (1949), Simpson (1944, 1949) and Zeuner (1950), for the times required for the production of new species, are valid. These estimates relate to periods of the order of magnitude of a half-million years for the most rapid rate of speciation. Under the circumstances we may say only that the Neo-Darwinian theory is still a theory based on incomplete evidences, and even that the masterly treatment of the theory by Simpson (1944) does not provide the missing evidences so much as to try to explain why they may not be obtained. This is a kind of negative evidence and such evidences may be dangerous.

It is possible then to say "not proven" for the Neo-Darwinian theory and to hold alternative views in regard to the evolutionary mechanisms operating even in highly specialized animals. Thus Goldschmidt (1940, 1952) categorically denies the theory of evolution by subspeciation only and claims that speciation and the evolution of the higher categories involve processes and mechanisms of a very different nature. Clark (1930) has

assumed a period of macroevolutionary changes in the early stages of animal evolution, though still upholding a monophyletic origin. This diversity of viewpoint is wholesome for no theory which is inadequately documented should be universally accepted, and this is true notwithstanding the fact that the Neo-deVrisian views of Goldschmidt and of Clark are also no more than theories.

But it is not specialized evolution that is to be discussed here but rather that part of the theory of evolution which relates to the remote beginnings and to the periods preceding the known fossil record which are concerned with what may be primitive rather than specialized evolution. The information available in regard to primitive evolution is indirect but even so it differs in degree rather than in kind from the information available in regard to the evolution of the last 500,000,000 years. In each case we try to judge the mechanisms and processes by their presumed results, for the nature of any process must be so judged.

A Brief Sketch of a Theory of Primitive Evolution

A complete theory of animal evolution must concern itself with all the kinds of mechanisms and all the periods of their evolution, viz.,

1. The beginnings of animal life and especially whether they were mono- or polyphyletic.
2. The probable nature of the first animals and their primitive reproductive and evolutionary mechanisms.
3. The nature of organisms and evolutionary processes during the long period in which mitotic and meiotic mechanisms of cell division were evolved and in which sexual reproduction gradually became predominant.
4. The nature of organisms and evolutionary processes during the last half-billion years, a period characterized by specialized evolutionary mechanisms and specialized organisms.

Current theories relate mainly to the last quarter of the probable period of life on earth and often only to a small fragment of that. Let us take up these periods in order and attend especially to the earlier ones.

1. *The beginnings*

It is not our intention to elaborate on the details of the presumed biochemical and biophysical processes which were concerned in the development of the first living protoplasmic systems. These topics have been discussed extensively by Alexander (1948), Blum (1951), Breder (1942), Horowitz (1945), Oparin (1938) and others. We will concern ourselves only with a matter which Breder in particular discusses, viz., the origin of life by natural chemical evolution or by rare accident.

Breder clearly contrasts the "rare accident" theory of life's origin with the natural chemical evolution theory. By common consent a rare accident is a highly improbable event. If the now generally accepted view that all animals are genetically related be critically examined it will be found to lead inevitably to the "rare accident" theory. But it is difficult to conceive of conditions of such an extremely localized character as to have produced but a single speck of original living protoplasm in two billion years. Furthermore from this minute speck all subsequent forms of life were supposed to be evolved and thus life began in a kind of perfect experiment, succeeding at once and producing a speck of protoplasm with almost unlimited capacities for growth, development and evolutionary diversification. Not only is this theory improbable in regard to the mechanism of life's origin but there is nothing in our observation of hereditary mechanisms today or in the results of evolution for 500,000,000 years to indicate that *any* known protoplasmic system has unlimited capacities for evolutionary diversification. Today after a thousand millon years or more of evolutionary selection and fixation

there is much wastage of protoplasmic materials concerned in evolutionary processes while the main patterns of organization are perpetuated. To assume that the rare-accident, perfect-experiment theory is correct is to deny all the laws of probability associated with it. On the contrary we believe that life arose as a result of the operation of processes which took place in accordance with the natural chemical evolution of elements and compounds and that its origin occurred whenever and wherever suitable conditions existed.

If this may be true, then, as a consequence, true genetic relationship would occur only among those organisms which actually did descend from common ancestry. In other words, contrary to the current belief in a strictly monophyletic origin of life for the correctness of which there is no direct proof, life probably arose polyphyletically, and the general similarity of all protoplasmic systems is not a proof of true genetic relationship. Rather these general resemblances (as many other morphological and physiological agreements) are fundamentally convergent and represent only the minimum "conditions of existence" required of living systems.

At this point it is natural to consider briefly the kinds of evidence which seem generally to have been responsible for the current belief in a strictly monophyletic origin of all life. Perhaps the most striking of the similarities among living things, which naturally incline biologists to the assumption of monophyletic origin and true genetic relationships among all organisms, is the occurrence of mitosis and meiosis on a fundamentally similar pattern in so many kinds of organisms. It is evidently believed that these processes *must be* monophyletic. However, there are many considerations which indicate that this may not be the correct conclusion and certainly is not the only possible interpretation of the facts. We list several of these considerations here.

(1) The mitotic and meiotic processes of cell division are exceedingly complex and probably required long periods of time, perhaps of the order of magnitude of hundreds of millions of years, for their evolution. Prior to the evolution of these mechanisms there were no such similarities among protoplasmic systems.

(2) The mitotic and meiotic mechanisms are not only complex but variable in their natures in regard to many of their typical features, e.g., intra- or extranuclear spindle; presence or absence of centrioles; one- or two-division meiosis; meiosis associated with sporulation or gametogenesis, etc.

(3) There are hardly any structural characters of animals which are not convergent in some groups, and the mitotic and meiotic mechanisms therefore differ in degree rather than in kind in the amounts of convergence shown when compared with other structural characters. Thus such major features of animal organization as symmetry, number of germ layers, presence of exoskeletons, paired jointed appendages, wings and winglessness, gills, camera-type eye, four-chambered heart, constant body temperature, and presence of hemoglobin, appear to be convergent in one or more animal groups.

Under the circumstances the widespread occurrence of what is called mitosis or meiosis is no proof of real genetic relationship among all such organisms. On the contrary the very existence of such mechanisms in organisms otherwise so diverse as Protista, Metazoa, and Metaphyta is strongly suggestive of convergence and may thus be interpreted in agreement with the theory of the strictly polyphyletic origins of the major groups of organisms.

2. *The nature of the first animals and their reproductive and evolutionary mechanisms*

There may be no free-living organisms today whose ancestral origins extend back less than 500,000,000 or a billion years. This being the case, the likelihood

that any such organisms are truly primitive is small. All have evolved to some extent and many to relatively great extents. What then of the nature of primitive organisms? It is a difficult task for us to conceive of truly primitive ancestral organisms when all that we may actually observe now have been the products of a billion years of evolutionary selection and fixation. But let us try, realizing that (1) mitosis and meiosis, and (2) sexual reproduction, are in no sense primitive processes.

The minimum stuff which was possessed of the barest properties of life was a protoplasmic system capable of metabolism, growth, and reproduction. We cannot certainly say whether this was a one major component system (nucleoplasm or cytoplasm) or a two-component system having both kinds of protoplasm. For our purpose it is sufficient to take up the story with a *primitive* two-component system, of *minimum* organization. There were probably no precise relations between the amounts of cytoplasm and nucleoplasm, no definite nuclei, nor chromosomes nor mitotic spindles nor meiotic divisions. This was the premitotic and presexual phase of evolution. Growth and reproducton were irregular and there was no precise timing between the growth and reproductive rates of the two major components. As a consequence variability was relatively great, by modern standards, and heritability or reproducibility most imperfect. Evolution was rapid and characterized more by change than by the conservation of change. In fact the greatest problem which such primitive organisms had to solve was the problem of *stabilizing a reaction norm* (Schmalhausen, 1949), in such organisms as did find favorable circumstances for living, without the benefit of a mitotic mechanism for cell division. The probability is that many of these organisms evolved themselves right out of existence because, while apparently trying all things, they had no effective machinery for holding fast to that which was good. And it was in this early period of primitive life that we believe the major evolutionary diversification in any line of descent took place—truly "explosive" evolution—considering the magnitude of the steps taken, though rapid evolution no doubt extended even into the next period during which the mitotic and meiotic mechanisms were being gradually evolved and perfected.

Obviously, the organisms of the premitotic period had no such precise reproductive mechanisms as are now concerned in the typical haploid-diploid alternation of chromosome numbers. Were they haploid in the sense that they had only one set of determiners or protogenes? In the absence of a mitotic mechanism which normally times the cleavage of the cell to follow the duplication of the genes it would be too much to expect that a haploid constitution could be characteristic of these organisms or could be maintained by the reproductive machinery at their command. Furthermore a haploid genome, as we know it now, is the barest minimum of determiners necessary for life in animals and it is quite unlikely that primitive protoplasm could have long survived without reserves, on such a haploid level.

It would seem, then, that the original protoplasm must have been truly primitive, relatively unorganized, but in a sense "polyploid" rather than diploid or haploid, that is, with variable numbers and multiples of protogenes of each kind, irregularly distributed throughout the mass of cytoplasm. Were these organisms Protozoa or Metazoa? By current standards they were unlike either. It would perhaps be better to refer to them as *Prozoa* for they were sufficiently unlike most existing Protozoa and Metazoa to warrant being distinguished from both. Moreover it is at least as likely that Metazoa evolved from those primitive Prozoa before Protozoa as afterwards for the theory of the evolution of Metazoa from colonial Protozoa has but the scantiest evidence

to support it. On the contrary primitive Metazoa do produce unicellular products even today—in their amebocytes, leucocytes, germ balls and gametes and the primitive processes just mentioned (not including gamete formation) could have occurred in the early Metazoa, as they still do in their modern descendants.

3. *The age of the evolution of mitosis, meiosis, and sexual reproduction*

Overlapping with the preceding period came a period in which definite steps were taken in the direction of perfecting the *reproductive* processes—so that it would be possible for an adapted organism in favorable circumstances to duplicate itself, to perpetuate its main pattern of organization, to "hold fast to that which was good." This problem was solved by the development of the machinery of mitosis, perhaps by the most complicated of all evolutionary sequences. Mitosis is an exceedingly complex process (Schrader, 1944; White, 1942), and the evolution of chromosomes, spindles, and a coordinatng mechanism to integrate the duplication and movement of chromosomes, and the division of the cytosome, must have required a relatively great period of time of the order of magnitude of hundreds of millions of years.

If mitosis required so long a period of evolution what kept it progressing? How could natural selection act to favor the early stages of this process and carry it on to completion? In my view every step in the development of this mechanism would have conferred some benefits on the organisms taking such steps. Thus any development which made it possible for the offspring to resemble more closely the parent would confer benefits on organisms living under favorable circumstances. The development of chromosomes helped to coordinate the duplication of the protogenes; the development of a spindle resulted in a more equal distribution of the duplicated protogenes, and the control of the timing of chromosome and cytoplasmic divisions gave similar daughter cells in the progeny. The final achievement of mitosis was a great advantage to these organisms for it guaranteed to all parts of the organism a similar genetic constitution. Only after this was achieved could a primitive organism regularly and normally

(a) Regenerate any missing part
(b) Reproduce asexually from one or more parts of the body.

Thus regeneration and asexual reproduction in primitive many-celled organisms, highly similar and correlated capacities and processes, were directly dependent on the perfection of the mechanism of mitosis, and we must look to *asexual reproduction* rather than sexual reproduction for the explanation of the evolution of mitosis.

It is customary in these times to consider asexual reproduction of no genetic or evolutionary significance. Even the current definitions of species (Dobzhansky, Mayr) are inapplicable to asexually reproducing organisms.[1] This results from a loss of perspective and from too great focussing on micro-evolution in highly specialized organisms. Current students of evolution are inclined to consider asexual reproduction or even parthenogenesis as processes leading to "blind alleys" but this also is due to some lack of perspective. When the whole story of the evolution of evolutionary mechanisms is considered we see that this view is quite incorrect, for the evidences cited indicate:

(1) That asexual reproduction was the only reproductive process which perpetu-

[1] Dobzhansky (1937). A species is "that stage of evolutionary process at which the once actually or potentially interbreeding array of forms becomes segregated in two or more separate arrays which are physiologically incapable of interbreeding."

Mayr (1942): "Species are groups of actually or potentially interbreeding natural populations, which are reproductively isolated from other such groups."

ated life for the very long period during which sexual reproduction was evolving.

(2) That major organic diversification probably occurred as it still occurs under asexual reproduction in relatively primitive organisms.

(3) That asexual reproduction could have evolved and become perfected only through the associated development of the mechanisms of mitosis.

(4) That without asexual reproduction it is highly unlikely that there would have been any evolution of sexual reproduction.

In this connection the observations of Berrill (1948, 1949) on coelenterates are of great interest and importance. In such colonial hydroids as the common species of Obelia, both kinds of reproduction occur and there is a relatively great amount of plasticity associated with each. Thus the products of budding in *Obelia articulata* and *O. geniculata* include (1) hydranths, (2) gonangia, (3) terminal gonangial buds, and (4) medusae. In Scyphomedusae the sexual reproduction is highly variable inasmuch as the size of the eggs and the course of ontogeny are both exceedingly varied. Thus it is that, even after some hundreds of millions of years, the reproductive processes in these Coelenterata retain a high degree of plasticity and variability as would be expected of relatively primitive organisms. But of the two kinds of reproduction, it is the asexual method which is directly responsible for the greater amount of organic diversification revealed in the polymorphism of the hydroid colonies. And this variability occurs in spite of the development of mitotic mechanisms of cell division and of the resulting same genomes in all cells of the bodies of all types of individuals descended by asexual reproduction from a single zygote.

If the evolution of mitosis may have required such a long period of time, what about meiosis? The transformation of mitosis into meiosis seems a relatively simple process for organisms which evolved (probably polyphyletically) a mitotic mechanism out of none. Thus the typical differences between mitosis and meiosis are two: (a) Synapsis in preparation for the first meiotic division, (b) Failure of chromosomal duplication in the second meiotic division. These principle differences are usually associated with tetrad formation and genetic reduction, processes which have come to play such vital roles in sexual reproduction and Mendelian inheritance. Perfected as these processes now are, in the beginning they were undoubtedly much simpler.

Even in existing species a host of variations and permutations of mitotic and meiotic processes have been described by Cleveland (1949, 1950, 1951) in recent years. Though these processes are now being studied in parasitic and specialized organisms they appear to reveal how the mitotic and meiotic processes are interrelated and how they may have evolved. It is of special importance that meiosis and gametogenesis seem to have evolved separately and become associated together later. Thus meiosis may still occur in asexual reproduction as in sporulation and be lacking from sexual reproduction as in diploid or polyploid parthenogenesis. It is advisable therefore to define sexual reproduction as reproduction involving gamete formation (with or without fertilization) and to distinguish it from asexual reproduction which does not involve gamete formation.

In this connection it may be well to clarify the nature of the process of parthenogenesis. It is described in some of our principal texts (Wilson, 1934; Darlington, 1939; White, 1945) as "asexual" and thus placed in the same general category as budding and fission. But the very authorities who do this, provide us with the evidences that parthenogenesis is derived from normal sexual reproduction and bears no significant resemblance to the processes of budding and fission (Boyden, 1950).

Let us consider the haploid parthenogenesis of the common honey bee. "All

eggs are reduced and haploid, having undergone typical gametogenesis. Such eggs are produced normally only in the ovaries of the queen. Assuming the marriage flight has occurred, most of these eggs will be fertilized and produce females, mostly workers. But some of these eggs may remain unfertilized and develop into drones or males. Whether any particular egg shall be fertilized or not depends upon the physiological operation of the female machinery which allows sperm access to the eggs, or denies it. Thus the sex of the offspring is determined. To recapitulate: *female* gametes, produced in *female* gonads, and stored in *female* accessory organs are either fertilized or not, depending upon the operation of the *female* reproductive machinery, which has been supplied previously with *male* gametes. If the eggs are fertilized they become *females,* if they remain unfertilized they become *males.* There is not an asexual atom in this whole procedure!" Boyden, 1950).

4. *The age of stabilization*

The last 500,000,000 years show no revolutionary changes in either the mechanisms of evolution or the products of these mechanisms. Every phylum likely to leave fossils is represented in the early Paleozoic rocks by species which obviously have a place in the phyla now recognized. Thus there have been no new phyla in four or five hundred million years and this period is characterized by *the conservation of change* as much as by the changes themselves.

Of evolution there has been a great deal along the lines of speciation and adaptive radiation but the fundamental patterns have been maintained by the conservative action of the mechanisms of asexual and sexual reproduction. Classes, orders and families may have been produced by subspeciational processes as visualized by the Neo-Darwinians, or they may have been produced by greater steps, or by both processes—the evidences are inconclusive. In support of the Neo-deVrisian theory are the data of the distributions of genera by size, the so-called hollow curves of Willis (1922, 1940). The simplest explanation for the general preponderance of monotypic genera is that the new species are often produced by mutational steps large enough to be reckoned as distinct genera. Also on the continuous evolution, the Neo-Darwinian, view there must have been a relatively enormous amount of extinction of animal types to produce the gaps between 400 different orders of animals, living or extinct. It is difficult to conceive how this enormous amount of extinction of connecting types could have occurred on the Neo-Darwinian theory or any other which assumes that all evolution is subspeciational.

Characterization of the Great Stages of Evolutionary History

We may briefly characterize the great stages of evolutionary history as follows (see figure 1):

1. An age of natural chemical evolution of inorganic and organic compounds culminating in the production and sterile storage of several kinds of carbohydrates, fats and even proteins. A very long period of time was probably involved, perhaps of the order of magnitude of a billion years.

2. An age during which the polyphyletic origins of living protoplasms occurred. Conditions were suitable in a number of different localities and at somewhat different times for the origin of living protoplasm. The period of time involved was probably much less than the preceding age and perhaps of the order of magnitude of some millions of years.

3. The unstable age, presexual and premitotic, followed. During this period of time there was a gradual evolution of mitotic and meiotic mechanisms and of sexual reproduction (gametogenesis). Evolution in general was rapid, even eruptive; there was much change and little conservation of change; such major

THE GREAT AGES OF EVOLUTION

Time in Millions of Years

Present	Era	Characteristics of Eras	Nature of Evolution
100	Cenozoic	Many phyla	Age of relative STABILIZATION
	Mesozoic		
500	Paleozoic	Fossils first abundant	Adaptive Radiation
1000	Pre-Cambrian	Fossils rare and uncertain	Reproduction More Perfect: Mostly Sexual
			Mitosis and Meiosis
1500		Heterotrophs and Autotrophs	Age of ERUPTIVE EVOLUTION
		Oldest Sedimentary Rocks	Reproduction Imperfect Asexual and Premitotic
2000		Heterotrophs	Age of POLYPHYLETIC ORIGINS
		Origins of Life	
2500		Accumulation of organic compounds	Age of NATURAL CHEMICAL EVOLUTION
3000		Sterile Earth	

FIG. 1. The great ages of evolution. The figure attempts to show the characteristics of the various eras of geological time and especially the relative durations and the natures of the evolutionary changes occurring during those times.

phyletic diversification as developed from monophyletic origins occurred at this time. Many ecologic niches were available but the organisms themselves may not have been as stable as the environments because reproductive mechanisms were relatively imperfect. The gradual development of these mechanisms led to the next age.

4. The age of relative stabilization, the final age, is characterized by sexual reproduction, mitotic and meiotic cell division, and conservation of the major basic patterns. Much gradual evolution occurred but not exclusively evolution by subspeciation. Evolution during this age was characterized as much by the conservation of change as by the changes themselves. Adaptive radiation, even "explosive" evolution in the sense of the relatively rapid divergence of the Mammalian orders, took place, but no new phyla were produced. This age is approximately equivalent to the last 500,000,000 years, and includes the Paleozoic, Mesozoic and Cenozoic Eras.

SUMMARY AND CONCLUSIONS

The subject of comparative evolution has not received the attention which its importance requires, and the amount of thought given to the evolution of evolutionary mechanisms from primitive to advanced states has been relatively small.

A survey of the state of knowledge on this topic seems to warrant the following summary and conclusions.

Direct evidences relating to the origins of life and the first half or three-quarters of animal evolution on earth are lacking. Consequently indirect evidences alone are available and reasonable speculation about these matters is warranted.

There are two general approaches to these problems:

(1) The method of extrapolation from the evolutionary trends revealed by the known fossil records back to the presumed points of origin.

(2) The method of direct assumption regarding the probable natures of early and primitive protoplasmic systems on the basis of a natural chemical evolution of elements, compounds and protoplasmic systems.

The method of extrapolation has failed thus far to permit of any decisive interpretation of primitive animal evolution for it has been found to be incapable of deciding (1) when animal life first appeared, (2) whether primitive evolution was eruptive or gradual and even (3) whether the evolution of animal life was mono- or polyphyletic. The method of extrapolation possesses serious defects. (1) It is based on the relatively specialized processes and products of a billion or more years of evolutionary experimentation, selection and fixation, and is therefore incapable of revealing primitive processes. (2) It tends to ignore the relatively primitive genetic, reproductive and evolutionary mechanisms still in existence today, such as the asexual reproduction of the simpler protists, plants, and animals. (3) It lacks perspective generally assuming that there has been no evolution of evolutionary mechanisms in one or two billion years. On the other hand the method of direct assumptions is based on indirect evidences and is admittedly largely speculative. This does not mean that the conclusions reached by it are necessarily less true than those reached by extrapolation from the known to the unknown where the processes concerned in the unknown stages of evolution were significantly different from those now or recently operating.

On the basis of the probabilities concerned we have reached the following tentative conclusions in regard to primitive evolution.

(1) Life originated as a result of the natural chemical evolution of organic compounds, polyphyletically.

(2) Primitive protoplasmic systems were as simple as conceivable, viz. heterotrophic, asexual and premitotic, "polyploid."

(3) Primitive evolution was eruptive, since the mechanisms of reduplication of protoplasmic systems and their component parts were imperfect.

(4) A long period of time was required for the development of mitotic mechanisms but a relatively slight modification of these produced meiosis, probably polyphyletically.

(5) Asexual reproduction has probably played a very significant role in primitive evolution and is a neglected factor in current evolutionary theory.

(6) Sexual reproduction and gametogenesis evolved to some extent independently of meiosis, converging subsequently to become highly correlated with each other.

(7) Finally, no amount of intensive study of shifting gene frequencies in the highly specialized cross-fertilizing animals of today will solve the problems of the nature of primitive evolution because the mechanisms concerned in the two ages of evolution were significantly different.

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EVOLUTIONARY PATTERNS OF ANTIGENIC SUBSTANCES OF THE BLOOD CORPUSCLES IN COLUMBIDAE [1,2]

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INTRODUCTION

Previous reports from this laboratory have described the identification of antigenic substances of the red blood corpuscles which distinguish a species of doves from another. The behavior of these antigenic substances, in backcrosses of the species and backcross hybrids to one or the other of the parental species, has been as expected if the respective antigenic substances were hereditary. The object of this paper is to show whether the respective antigenic substances, specific to one species in contrast to another, may be found in other related species.

EXPLANATORY REMARKS AND EXPERIMENTAL PROCEDURE

Species hybrids have been obtained from mating Pearlneck (*Streptopelia chinensis*) males to female Ring doves (*St. risoria*), and backcross hybrids have been obtained from matings of the species hybrid and selected backcross hybrid to Ring dove. In the progeny from such matings it has been possible to obtain in unit form the majority, if not all, of the antigenic characters of the red blood corpuscles, or cells, specific to Pearlneck in comparison with Ring dove. Thus, nine or ten cellular characters peculiar to Pearlneck in comparison with Ring Dove have been obtained in presumed unit form, as previously described (Irwin, 1939). These have been called d-1, d-2, d-3, d-4, d-5, d-6, d-7, d-9, d-11, and d-12, the letter "d" indicating "dove." It should be stated that there is considerable antigenic resemblance, probably identity, between the d-4 and d-9 cellular characters of Pearlneck, and, instead of d-7 being a single substance, the blood cells carrying it seemingly contained the d-3 as well as the d-7 substance.

Each of these specific cellular antigens of Pearlneck, with but rare exceptions, has behaved as if it were produced by a single gene. A definite fractionation of the d-4 substance has been observed (Irwin, 1949), presumably as a result of crossing over of linked genes, and it seems plausible to propose that other specific substances of Pearlneck than d-4 may result from the joint action of two or more linked genes. Since each of these specific antigenic characters of Pearlneck has segregated independently of the others, it appears that the causative genes are located on independent chromosomes.

The details of the technics required for the recognition of these antigenic characters have been described in detail elsewhere (Irwin and Cole, 1936; Irwin, Cole and Gordon, 1936; Irwin, 1939). The technics used in the tests reported in this paper involved the same kind of procedures as those previously described, the only difference being that antisera to the cells of many different species of pigeons and doves were used. These antisera were produced in rabbits by immunizations with the blood cells of each of the various species. Since the anti-

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serum to one species would usually agglutinate the cells of many or all other species to the same dilution that obtained for the homologous cells (*i.e.*, those used in the immunization), no differentiation of the cells of the different species was possible by this test. However, when the antiserum to one species, as to *Streptopelia capicola* in table 1, was mixed at an appropriate dilution (usually 1 part antiserum to 60 parts of physiological saline isotonic for the cells of birds) with an excess of washed erythrocytes of Ring dove, the antiserum so "absorbed" became a "test fluid" or "reagent" which would be reactive with the blood cells of *capicola*, and of another species, as Pearlneck, only if cellular antigens were shared by that species and *capicola* to the exclusion of Ring dove. In essence, the phenotype (the complex of cellular antigens) of *capicola* was transferred to an outside medium, *i.e.*, to the antibodies of the antiserum. The antibodies are complementary to, or may be visualized as being the counterparts of, the respective antigenic substances of the immunizing cells and, following absorptions with the cells of another species, the specificity of the phenotype may be altered at will. Absorption of this antiserum to *capicola* with Ring dove cells would leave antibodies with one kind of specificity, and absorption with the cells of another species (as Pearlneck) would leave antibodies of a different specificity, if the antigenic composition of the two kinds of absorbing cells were different.

It is clearly impossible in this paper to attempt to explain the many ramifications of the technics of antibody-absorption. However, there are certain concepts of the relationships of cellular antigens in different species which may be deduced from the results of tests which follow the antibody-absorption. For example, if an antigenic substance "A" were recognized in two species, there are two relationships possible—"A" may be identical (homologous) in the two species, or it may be a similar substance but not identical. If the antiserum to each species were absorbed by the cells of the other, and antibodies for this A substance were removed in each case, it would be concluded that the antigen A in the two species was indistinguishable and presumably identical (or homologous). But if, following the absorption of each antiserum by the cells of the other, antibodies remained which would interact with cells containing the A substance of the species against which the antiserum was produced, the conclusion would be that the A substances were related antigenically, but were not identical. An early example of the recognition that the A and B cellular antigens of man are indistinguishable and presumably identical with the A and B cellular substances as they occur in the anthropoid apes is given by Landsteiner and Miller (1925).

The species against whose blood cells antisera have been produced and used in these tests are members of various categories of the family Columbidae. Representatives of the following species have been tested within the genus Streptopelia: *capicola*, the Cape turtle dove; *dussumieri*, the Philippine turtle dove; *humilis*, the dwarf turtle dove; *chinensis*, or Pearlneck; *risoria*, or Ring dove; *semitorquata*, the African turtle dove; and *senegalensis*, the Senegal or Palm dove. Tests have also been made with antisera to various species of the genus Columba: *fasciata*, the band-tailed pigeon; *flavirostris*, the red-billed pigeon; *guinea*, the triangular-spotted pigeon; *janthina; leucocephala*, the white-crowned pigeon; *livia*, the domesticated form of the common pigeon; *maculosa*, the spot-wing pigeon; *oenas*, the European stock pigeon; *palumbus; picazuro*, the Picazuro pigeon; and *rufina*, the rufous pigeon. Also, antisera to the following *six* species representing five other genera were available for these tests: *Ocyphaps lophotes*, the Australian crested dove; *Phaps chalcoptera*, the bronze wing dove; *Phaps elegans*, the brush bronze wing dove; *Zenaidura*

macroura, the mourning dove; *Zenaida aurita,* the Martinique dove; and *Melopelia leucoptera,* the white-wing dove.

Representatives of the above species were purchased from various dealers in the U. S.; one species (*St. semitorquata*) was obtained from shipments from Africa by an importer, and a few representatives of the *Columba picazuro* and several of the *St. dussumieri* were sent directly from Argentina and the Philippine Islands, respectively. Only one bird of *C. janthina* was available for these tests. In the other species, several or many individuals have been tested at one time or another, and with the observed variation between individuals at a minimum, unless otherwise stated.

It is recognized at the outset that the following comments on species relationships are valid only in so far as the reactions of the individuals tested within any single species reflect the antigenic complex of the whole species.

Specific Antigenic Characters of Pearlneck, in Contrast to Ring Dove, in Other Related Species

Having obtained in presumed single form the majority, if not all, of the antigenic components of the erythrocytes which distinguish Pearlneck from Ring dove, it becomes possible to use these as "tester substances" to determine whether these specific substances of Pearlneck are shared, in whole or in part, with other species. Since these specific substances have been shown to be hereditary, the assumption would appear reasonable that the same or related substances in other species to those in Pearlneck would also be gene determined, and deductions from the interrelationships of the cellular antigens would thus provide a basis as to whether homologous or related genes for these particular substances of Pearlneck were present in other species under test. (The term "homologous" in this paper is used primarily in the sense that the antigenic substances in two species may be indistinguishable by immunological technics, and therefore seemingly are identical or homologous. If the antigenic substances in the two species are homologous, the causative gene or genes in the two species may be spoken of as also being homologous, since their respective products are indistinguishable.) Any such deductions are based on the assumptions (a) that in other species either an identical or similar substance to an antigenic character specific to Pearlneck would be gene-determined, and (b) that, if two or more homologous or similar antigenic substances to those of Pearlneck were present in another species, the causative genes would be independent, as in Pearlneck. This latter assumption might well have exceptions, as will be discussed elsewhere.

The general procedure used for the detection of similar substances in other species to those specific to Pearlneck rests on the premise that the antiserum to any other species, following absorption with Ring dove cells, becomes a reagent which will be reactive with the cells of any species bearing the same or related antigenic components to those of Pearlneck. Thus, since it was observed (unpublished data) that anti-Pearlneck serum, absorbed with Ring dove cells, agglutinated in varying dilutions the cells of all the species used in these tests, it would be expected that antisera prepared against each species, and similarly absorbed with the cells of Ring dove, would be reactive with Pearlneck cells and therefore with one or more of the individual constituents specific to Pearlneck, in contrast to Ring dove. The results obtained by using the respective antisera to the various species in such tests are given in table 1. Each antiserum was absorbed with Ring dove cells and tested with the cells of Pearlneck and of backcross birds carrying the unit antigens of Pearlneck, as is given in table 1.

Particular attention should be called to the presumed specificity of the interaction

TABLE 1. *Tests for the presence, in the antisera against various species, of antibodies to the respective specific antigenic substances of Pearlneck, as compared to Ring Dove*

Antisera	Reactions of the respective reagents prepared from the antisera to each of the species as listed, when tested with the blood cells carrying the respective unit substances specific to Pearlneck in contrast to Ring Dove													Summary of the agglutination of the specific antigenic characters of Pearlneck
	Absorbed by cells of	Test cells												
		R.D.	P.N.	d-1	d-2	d-3	d-4	d-5	d-6	d-7	d-9	d-11	d-12	
Streptopelia														
capicola	Ring Dove	0	++	+	0	±	0	0	0	0	0	+	−	d-1, d-3, d-11
dussumieri	Ring Dove	0	++	+	±	±	+	?	?	?	+	+	±	d-1, d-2, d-3, d-4, d-11, d-12
humilis	Ring Dove	0	++	++	?	?	+	?	?	(2)	+	+	±	d-1, d-4, d-11, d-12
orientalis	Ring Dove	0	++	++	(2)	0	+	0	?	0	±	++	0	d-1, d-4, d-11
Pearlneck	Ring Dove	0	C	++	++	++	++	++	+	++	++	C	+	d-1 to d-12 inclusive
Ring Dove	Ring Dove	0	0	−	−	−	−	−	−	−	−	−	−	none
semitorquata	Ring Dove	0	0, ±	−	−	0	0	0	−	−	−	−	−	none
senegalensis	Ring Dove	0	C	++	+	+	++	++	±	+	+	++	+	d-1 to d-12 inclusive
Columba														
fasciata	Ring Dove	0	++	?	+	0	0	±	?	±	0	±	?	d-2, d-5, d-7, d-11
flavirostris	Ring Dove	0	+	±	±	0	?	±	±	±	?	?	±	d-1, d-2, d-5, d-6, d-7, d-12
guinea	Ring Dove	0	++	+	?	0	±	0	0	0	−	++	0	d-1, d-4, d-11
janthina	Ring Dove	0	++	0	0	0	0	0	±	±	0	++	0	d-6, d-7, d-11
leucocephala	Ring Dove	0	++	0	0	0	0	0	0	±	−	++	0	d-7, d-11
livia	Ring Dove	0	+	?	0	±	0	±	±	±	0	?	±	d-3, d-5, d-6, d-7, d-12
maculosa	Ring Dove	0	+	?	?	0	0	±	?	±	0	0	0	d-5, d-7
oenas	Ring Dove	0	++	±	0	0	0	?	0	±	0	++	±	d-1, d-7, d-11, d-12
palumbus	Ring Dove	0	++	+	0	0	0	?	±	0	0	+	−	d-1, d-6, d-11
picazuro	Ring Dove	0	++	0	++	0	±	0	0	±	+	++	0	d-2, d-4, d-7, d-11
rufina	Ring Dove	0	++	±	++	±	±	±	0	±	0	++	±	d-1, d-2, d-3, d-4, d-5, d-7, d-11, d-12
Aus. Crested	Ring Dove	0	++	++	0	0	0	0	0	?	0	?	−	d-1
Bronze Wing	Ring Dove	0	++	++	0	?	?	0	?	?	0	0	−	d-1
Brush Bronze Wing	Ring Dove	0	++	++	0	0	0	0	?	?	0	?	−	d-1
Martinique	Ring Dove	0	±	0	0	0	±	0	0	0	±	0	−	d-4
Mourning Dove	Ring Dove	0	±	±	?	?	+	?	?	?	±	?	−	d-1, d-4
White Wing	Ring Dove	0	++	+	0	0	?	?	?	?	±	?	−	d-1, d-9
Column 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15

Symbols: C = complete agglutination; ++ = very strong agglutination; + = strong agglutination; ± = definite but weak agglutination; 0 = no agglutination—at the first dilution of reagents and cells; (2) = no agglutination in the second dilution of the reagent-cell mixture; A dash (−) indicates that the combination of reagent and cells was not made. Questionable reactions are indicated by a question mark (?).

of the reagents, prepared by absorbing with Ring dove cells the antisera to the various species, with the cells of the backcross birds carrying any one of the unit-antigens specific to Pearlneck. By virtue of the successive matings to Ring dove, all these backcross birds carrying the respective "tester substances" of Pearlneck should have *all* the antigenic characters of Ring dove plus a *single* antigenic substance peculiar to Pearlneck. Individual differences in antigenic substances among Ring doves are certainly to be expected, but to date none have been noted at the dilution of antisera used in these tests (1:60 or higher). Each antiserum will of course possess a variety of antibodies, but the antibodies capable of reacting with the cells of the backcross birds, which bear all the complex of Ring dove antigens and an antigen peculiar to Pearlneck, will be only those reactive with the antigens of Ring dove or the substance peculiar to Pearlneck. Following absorption with Ring dove cells, the antiserum to any of these species will no longer contain antibodies to the antigens of Ring dove cells, and the reactivity with the cells of any of these backcross birds will be by virtue of an antibody induced against an antigenic substance of the given species which is either identical with, or related to, the particular antigen of Pearlneck contained in the cells of the backcross hybrid. Certainly each antiserum, other than those against Pearlneck and Ring dove, will have antibodies with specificities in addition to those shared with either Pearlneck or Ring dove, or both, but such antibodies presumably will have no capacity to interact with the cells of the backcross birds carrying the "tester substances" specific to Pearlneck. (There is one possible exception to this last general statement, in that the antiserum to the cells of the species hybrid (F_1—Pearlneck/Ring dove) contains antibodies against a "new" or "changed" specificity of the cells of the hybrid, the so-called "hybrid substance" (Irwin, 1939). If antigenic substances identical to or related to this hybrid-substance were present in the cells of other species—as has actually been observed but always if a substance is present which is at least related to a specific antigen of Pearlneck (as d-4 and d-11) with which a fraction of the hybrid substance is associated in the backcross hybrids—it is possible but not probable that the antiserum to such a species might contain antibodies to the hybrid substance but not to the specific Pearlneck substance. However, there is no evidence that the data and conclusions of this paper are complicated seriously by such antibodies.)

The expected variation in reactivity of different antisera to the cells of a single species was observed. For example, the reagents prepared from certain antisera to *humilis* did, and from others did not, agglutinate the cells with the d-1 and other antigens of Pearlneck. Presumably such differences in antibody content of the antisera are a reflection of the response of individual rabbits to immunization, as has been described (Irwin and Golden, 1942). Therefore the reactions which are recorded in table 1 are in most cases a summary of the tests of the reagents prepared individually from several antisera against each species. Thus, if only one antiserum of several against a species contained antibodies against a species specific substance of Pearlneck, the successful agglutination is the only one recorded in the table. If there was a question as to whether the agglutination was clearly expressed or not—*i.e.*, by microscopic inspection the cells were not distributed completely at random but neither were they definitely agglutinated in comparison with the absorbing cells—the reading is recorded in the table as doubtful (?). These questionable reactions, or at least the majority of them, should be considered as probably belonging with the reactions expressing lack of agglutination, rather than otherwise.

It should be recognized that other antisera to the various species might display additional reactivities to those listed in

table 1, but it is reasonable to believe that probably the majority of the potentially positive agglutinations of specific Pearlneck components have been observed. Furthermore, although a reagent prepared from an antiserum to a species may be reactive with one (or more) of the substances peculiar to Pearlneck, one cannot conclude that there is present in this species an antigenic substance identical (homologous) to that of Pearlneck. Neither can the degree of reactivity of the reagent-cell combination be considered as more than suggestive of the quantitative relationships of the respective antigens. For example, weak agglutination by any of the reagents of cells containing a Pearlneck component which with a Pearlneck reagent was always strongly reactive, as d-4, d-5 or d-11, would suggest strongly that only a related substance was present in the species whose antiserum gave such a weak reaction.

In examining the data of table 1, it should be kept in mind that if, as was previously stated, d-4 and d-9 are serologically equivalent, or nearly so, identical reactions should be recorded for them in the respective combinations with the various reagents. This condition has met with but two exceptions—differential reactions were obtained for d-4 and d-9 with the reagent from *rufina* antiserum (columns 8 and 12) and with the reagent from the antiserum to the White wing dove a questionable reaction was noted with d-4 cells, and weak but definite agglutination with d-9. Despite these discrepancies, these two substances are treated as one (d-4) in the summary of the reactions for each species which is given in column 15 of the table. Similarly, if d-7 was made up of d-3 as well as d-7, all reagents reactive with d-3 should also be reactive with d-7, but not the reverse. There is one exception to this latter criterion involving the reagent from antiserum to *capicola,* and possibly another involving the questionable reaction of d-7 cells with the reagent from the antiserum to *dussumieri.* The reason for these exceptions is not entirely clear at the present writing.

From the reactions recorded in table 1, it may be seen that the antisera against each of the species listed in the table, following absorption with the cells of Ring dove, contained antibodies which agglutinated Pearlneck cells (column 4). These results represent the reciprocal substantiation of the reactivities of the cells of these different species with the reagent specific for Pearlneck specific substances (Pearlneck antiserum absorbed with Ring dove cells), as stated earlier. This latter reagent had usually agglutinated the blood cells of *semitorquata, flavirostris* and *maculosa* only at the first or second dilution (1 : 90 or 1 : 180), those of *fasciata* and *livia* at the third or fourth dilution (1 : 360 or 1 : 720), and those of the other species at higher dilutions. (The details of these reactions will be furnished upon request.)

It may be noted in table 1 that the reagent prepared from the antiserum to *capicola* reacted with Pearlneck cells and also with the Pearlneck specific substances d-1, d-3 and d-11 (summary in column 15). From these reactions it would appear that *capicola* shared with Pearlneck, in contrast with Ring dove, at least a part if not all of the antigenic substances d-1, d-3, and d-11. Since there was at least a resemblance in the products of the respective causative genes in Pearlneck and *capicola,* the genes themselves in these two species would be expected to be alike to the extent that their products were related, as determined from the immunological tests alone. It has previously been shown that the predictions of the genetic relationships of antigenic characters of Pearlneck and Senegal, as demonstrated by immunological tests paralleling those of table 1, were actually fulfilled following the required genetic tests (Irwin and Cole, 1940; Irwin, 1949), thereby adding to the confidence placed in the accuracy of the interpretation of the present immunological

tests. Thus, it would be expected from the reactions listed in table 1, which show that there are antibodies in the antisera to both *humilis* and *orientalis* which are reactive with d-1 cells, that, if *capicola, humilis* and *orientalis* were hybridized with Ring dove and backcross hybrids could be obtained, a separation of antigenic substances related to, or identical with, d-1 of Pearlneck would be observed. Certain of these expected results are actually being obtained at present, and will be reported elsewhere.

The antiserum to a second species of Streptopelia, *dussumieri,* contained antibodies to d-1, d-2, d-3, d-4, d-11 and d-12, with questionable reactions to d-5, d-6 and d-7 (column 15). *If* both *capicola* and *dussumieri* shared the same fractions of d-1, d-3 and d-11 equally with Pearlneck, the deduction would appear to be justified that *dussumieri* and Pearlneck are more closely related in the antigenic substances which are specific to Pearlneck —not in Ring dove—than are *capicola* and Pearlneck. This of course does not mean that Pearlneck and *dussumieri* are more closely related than are *capicola* and Pearlneck in the antigenic substances other than these three. Furthermore, the reactions which are presented in the table do not of themselves provide information as to whether the specific substances of Pearlneck, which may be present in other species, are homologous or only related substances. Further tests are required to provide that kind of information.

The two extremes of reactivity—antibodies to all the specific substances of Pearlneck on one hand, and to practically none, if to any at all, on the other—are represented in two other species of Streptopelia, *senegalensis* and *semitorquata.* That is, the antiserum to *senegalensis* contained antibodies to each of the specific substances of Pearlneck, as previously described (Irwin and Cole, 1940; Irwin, 1949), and that to *semitorquata* contained only faintly reactive antibodies to Pearlneck cells. This reaction was so weak that the reagent from *semitorquata* antiserum was not tested with all the antigenic substances peculiar to Pearlneck, as may be noted in table 1. Each of the species other than semitorquata, however, judging from the reactions given in table 1, contains one or more antigenic substances related to those specific to Pearlneck.

It should be pointed out that a greater number of substances which bear some sort of relationship to those of Pearlneck, in one species than in another, does not necessarily indicate a closer relationship of the first species with Pearlneck. Certainly Senegal (*senegalensis*) is very closely related to Pearlneck, since in previous tests it has been found to contain substances either homologous with or related to each of nine of the Pearlneck specific characters: four are homologous, or nearly so, and five are similar but not homologous in the two species (Irwin, 1949). But one should not infer that those species which by these tests share five or six related antigens with Pearlneck—as *flavirostris, livia,* and *rufina* are more closely related to Pearlneck, in contrast to Ring dove, than are other species which share a smaller number, as *capicola, guinea* and Mourning dove, for example. The quantitative relationships of the sharing of the antigenic substances should also be considered, in so far as they can be measured.

Thus, within the genus Columba, the reagent prepared from the antiserum to *flavirostris* reacted definitely with Pearlneck cells, and weakly with the substances d-1, d-2, d-5, d-6, d-7 and d-12. A comparable situation obtains for both *livia* and *maculosa,* in that the reagent from *livia* reacted weakly with d-3, d-5, d-6, d-7 and d-12, and that of *maculosa* with d-5 and d-7. Do these results imply that *flavirostris* and *livia* are more closely related to the specific substances of Pearlneck blood cells than is *maculosa?* There is no clear answer to such a question, and the evidence indicates that whatever this relationship to Pearlneck may be within each of these species, it is very slight.

Further immunological tests could be devised to ascertain the relative degree of similarity of these three species to the specific Pearlneck antigenic components. In so far as these have been done, they indicate that, on the basis of antibodies in a Pearlneck antiserum, the cellular antigens in *maculosa* related to those specific to Pearlneck are more numerous, or are in greater quantity, than those in *flavirostris*. That is, the cells of *maculosa* by absorption removed from Pearlneck antiserum the antibodies for itself and for the cells of *flavirostris,* whereas a similar absorption by the cells of *flavirostris* did not remove all the antibodies for the cells of *maculosa*. This is in direct contradistinction to the relationships given in table 1, but could be explained by assuming that the antibodies in the antisera to *maculosa* were not representative of all the antigens in *maculosa* related to the specific substances of Pearlneck. In the light of those considerations, it seems clear that the reactions given in table 1 do not of themselves provide a complete basis for assaying the relative relationships of any of the species to those cellular antigens of Pearlneck which distinguish it from Ring dove.

One of the most informative ways to examine the results of table 1 would be to list the appearance for each of the species of reactivity with the respective specific Pearlneck characters, as is given in column 15 of table 1. This listing in turn can be summarized to show the positive and also the negative (including those questionable) agglutinations of each of the specific substances of Pearlneck as they occur in the other species, as is given in table 2. It may be noted in this summary that each of the specific characters of Pearlneck, or a related character, is found in at least one other species of Streptopelia, and also in at least one species of Columba, usually in several. However, definite evidence for the presence of only two of the nine substances—d-1 and d-4—has been noted in the representatives of the genera other than Streptopelia and Columba. Certainly it can be generally concluded that a substance related to each of the known antigenic substances which distinguish Pearlneck from Ring dove may be found in other genera, as well as in one or more other species within the genus Streptopelia.

TABLE 2. *A summary of the presence or absence in the respective species of the antigenic characters related to or homologous with those specific to Pearlneck in contrast to Ring Dove*

Antigenic character	Streptopelia		Columba		Other genera		Totals	
	Present	Absent	Present	Absent	Present	Absent	Present	Absent
d-1	6	2	5	6	5	1	16	9
d-2	3	5	4	7	0	6	7	18
d-3	4	4	2	9	0	6	6	19
d-4	5	3	3	8	3	3	11	14
d-5	2	6	5	6	0	6	7	18
d-6	2	6	4	7	0	6	6	19
d-7	2	6	9	2	0	6	11	14
d-11	6	2	8	3	0	6	14	11
d-12	4	3	4	6	–	–	8	9

It is interesting to note, in the summary in table 2, that five of the nine Pearlneck characters have related substances in four or more—but not necessarily in the same species—of the eight species of Streptopelia. These are d-1, d-3, d-4, d-11 and d-12. Two of these, d-1 and d-11, appear in all the species of Streptopelia except Ring dove and *semitorquata;* both have related forms in five or more of the eleven species of Columba. The two antigens differ, however, in their distribution in the six species of other genera tested than Columba and Streptopelia in that in these

other species substances related to d-1, but none to d-11, have been demonstrated to be present.

Reactions have been noted which indicate the presence of both d-2 and d-3 in several species of Streptopelia other than Pearlneck. Both are present in four and two, respectively, of the eleven species of Columba, but not in any of the six species of the other genera.

Within the eight species of Streptopelia, d-5, d-6 and d-7 were detected in only one other species (Senegal) than Pearlneck, whereas each of these three antigens had a related substance in several species of Columba (in five, four and nine species, respectively), but not in the other genera tested. It is perhaps worthy of note that d-5 has an homologous substance, or nearly so, in Senegal (Irwin, 1949), but that there are only related substances, not identical, in Senegal to both d-6 and d-7. Certainly the type of reactions observed with each of these substances, d-5, d-6 and d-7, in combination with the reagents from the species of Columba would suggest that the relationship of the antigenic substance in these species, wherever the substance presumably was present at all, was a very distant one indeed to that of Pearlneck.

Substance d-12 was reactive with reagents made from antisera to several species in both Streptopelia and Columba, but tests for its presence were not made in the species of the other genera, nor with the reagent from antiserum to *C. palumbus*.

The d-4 character appears to resemble d-1 in being highly cosmopolitan, for these two antigens showed the widest distribution in the various genera. As has been stated, the reactions given in table 1 indicate that d-1, or a related substance, is present in six of the eight species of Streptopelia (in all but Ring dove and *semitorquata*), in five (*flavirostris, guinea, oenas, palumbus* and *rufina*) of the eleven species of Columba, and in five of the six species (in all but the Martinque dove) of genera other than Columba and Streptopelia. The d-4 substance is not quite as widespread in these 25 species as is d-1, being present in five (*dussumieri, humilis, orientalis, Pearlneck* and *Senegal*) species of Streptopelia, in three (*guinea, picazuro* and *rufina*) of the eight species of Columba, and in three species (Martinique, Mourning dove and White winged dove) of the other genera tested.

In the preceding discussion of the distribution of the antigenic substances specific to Pearlneck among the 25 species tested, the substitution of the causative genes for the antigenic substances themselves would provide a picture of the gene distribution in the various species. Since the environment seemingly has no effect upon the cellular antigens, the relationships revealed in this study may be considered as material from which the evolutionary pattern of the genes under study may be traced. To make this pattern as precise as possible, however, information is required as to whether the gene effects —and presumably therefore the causative genes—are homologous or related in the various species.

Identity or Similarity of the Substances in Other Species to Those of Pearlneck

The evidence which has been presented shows that each of the specific antigenic characters of Pearlneck, in contrast to Ring dove, has appeared in a related form in one or more other species, of the same or a related genus. A next step in assaying the relationships here described is to determine identity or non-identity to the antigenes in Pearlneck of these substances in the various species in which they appear. Critical tests to this end can be made by determining whether the cells of each species in combination with those of Ring dove will remove the antibodies from Pearlneck antiserum for any of the antigenic substances of Pearlneck. Thus, if the cells of *capicola* contain the antigenic substances d-1, d-3 and d-11 in homologous form to those of Pearlneck, they should by absorption of Pearlneck

antiserum remove the antibodies for these three Pearlneck substances. If this result were obtained, the conclusion would be that the respective substances in the two species (Pearlneck and *capicola*) were indistinguishable and therefore presumably identical. However, if absorption with the cells of *capicola* did not remove completely the antibodies for d-1, d-3 and d-11, as was actually found to be the case (column 3, table 3), the only valid conclusion is that there are related but not identical substances in *capicola* to d-1, d-3 and d-11 of Pearlneck. The questionable reaction of this reagent with the cells of d-12 may indicate (1) that *capicola* possesses a related or homologous substance to d-12, or (2) that there had been a non-specific absorption of the relatively weakly reactive antibody of Pearlneck antiserum for d-12.

The reactions of the cells of backcross hybrids, carrying the different substances peculiar to Pearlneck with the specific reangent (anti-Pearlneck serum absorbed with Ring dove cells), as given in column 2 of table 3, form the basis of comparison for the reactions with these cells of the reagents prepared by further double absorptions of Pearlneck antiserum with the cells of Ring dove and each of the various species. The reaction of d-12 with the reagent for specific Pearlneck characters was relatively weak, and it is possible that the antibodies to d-12 could be removed non-specifically, or by contact with a related but not identical substance. Hence there may be a question as to the interpretation to be placed on the lack of agglutination, or the questionable agglutinations, of cells carrying d-12 with certain reagents in table 3.

From the reactions given in table 1, it appeared that there were antibodies in the antisera to *dussumieri* to the d-1, d-2, d-3, d-4, d-11 and d-12 substances of Pearlneck, with questionable agglutinations of cells with d-5, d-6, and d-7. According to the reactivities of these unit substances following the use of the cells of *dussumieri* in absorption of Pearlneck antiserum (column 4 of table 3), there were questionable reactions with d-4, d-6 and d-7, indicating that substances at least closely related, *possibly homologous,* to these three were present in *dussumieri,* and that it possessed substances only related to d-1, d-2, d-3, d-11 and d-12 of Pearlneck, possibly to d-5.

Certain of the antisera against the cells of *humilis* have contained antibodies against d-1, d-4, d-11 and d-12 (table 1), whereas the cells of *humilis* by absorption (column 5, table 3) removed antibodies completely for only the d-4 substance, not for d-1, d-11 or d-12. Hence, an homologous substance to d-4 of Pearlneck appears to be present in *humilis,* but *humilis* contains substances only related to d-1, d-11 and d-12 of Pearlneck. A parallel situation exists in *orientalis,* in that there were antibodies in *orientalis* antisera reactive with d-1, d-4 and d-11 (table 1), but only the antibodies for d-4 cells were removed by the absorption of Pearlneck antiserum with *orientalis* corpuscles (column 6, table 3), not for d-1 and d-11. Thus both *humilis* and *orientalis* appear to share with Pearlneck an homologous substance, d-4, and substances related to d-1 and d-11. But whether the substances related to d-1 and d-11 in these two species are homologous or only related to each other cannot be inferred from these tests.

As was previously stated, the antiserum to Senegal contained antibodies to each of the specific antigens of Pearlneck, indicating that the cellular antigens of Senegal are either related to or homologous with those of Pearlneck, to the mutual exclusion of Ring dove. From the results given in table 3 (column 8), it may be seen that, following absorption of the Pearlneck antiserum with the cells of Senegal, there were only faint reactions of the reagent with the cells containing d-1 and d-7, stronger clumping of cells with d-2, d-6 and d-11, and either doubtful or no agglutination of the cells with d-3, d-4, d-5 and d-12. Hence Senegal contains antigenic substances which are

TABLE 3. *Tests to determine the homology or non-homology of cellular antigens in other species of Columbidae to the species-specific antigens of Pearlneck, in contrast to Ring Dove*

Cells tested	The agglutination of various cells as given with Pearlneck antiserum absorbed with Ring Dove cells (column 2), or with Ring Dove cells plus those of each of the various species as listed (columns 3 to 24 inclusive)																						
		capicola	dussumieri	humilis	orientalis	semitorquata	senegalensis	fasciata	flavirostris	guinea	leucocephala	livia	maculosa	oenas	picazuro	rufina	Aus. Crested	Bronze Wing	Brush Bronze Wing	Grayson	Martinique	Mourning dove	White Wing
Pearlneck	9	8	8	8	8	8	8	8	8	8			8		8	8	8	8	8	8	8	8	8
Ring Dove	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
d-1	6,7	++	++	++	++	++	±	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
d-2	4,5	++	++	++	++	++	+	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
d-3	4,5	+	±	+	+	?	0	+	++	±		+	+	−	+	+	+	+	++	++	++	++	+
d-4	2,3	+	?	0	0	±	0	±	±	±	+	+	+	?	?	±	±	+	+	?	±	?	?
d-5	6,7	++	++	++	++	++	0,?	+	++	++	++	++	++	++	++	++	+	++	++	++	++	++	++
d-6	3,4	±	?	±	0	±	+	+	±	+	++	+	+	+	+	±	±	±	+	±	+	+	+
d-7	4,5	±	?	+	+	?	±	+	+	±	+	+	+	±	±	+	±	±	++	+	+	++	++
d-11	8	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
d-12	1,2	?	±	±	±	−	0	±	+	+	±	?	±	?	+	±	0	0	0	−	±	?	?
Orientalis	7	++	±	+	0	++	++	++	++	++			+		++	++	++	++	++	++	++	++	++
guinea	6	++	−	++	+	++	++	++	++	0			++		++	++	++	++	++	++	++	++	++
Senegal	8	++	++	++	++	++	0	++	++	++			++		++	++	++	++	++	++	++	++	++
Column 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24

Symbols: Same as in table 1. The digits in column 2 represent the highest dilution of the reagent for specific Pearlneck components at which agglutination of the cells was visible. For example, the first dilution of the reagent-cell combination was at 1:90, and 1 = 90, 2 = 180, 3 = 360, ··· 9 = 23,040. If two digits are listed, as 6 and 7 opposite d-1, there was variation noted in the highest dilution.

indistinguishable from, and presumably identical to, the d-3, d-4, d-15 and d-12 of Pearlneck, and these have been obtained and identified in unit form in backcross offspring of the cross between Senegal and Ring dove (Irwin, 1949). Further, the antigens of Senegal related to d-2, d-6, d-11 and possibly to d-7 have also been identified as unit characters, and two substances related to d-1 have been noted which behave as if the causative genes in Senegal were linked. And since Pearlneck and Ring dove share very few, if any, cellular characters—therefore the causative genes—not also shared by Senegal (Irwin and Cole, 1940), it seems certain that, by this serological criterion, Pearlneck is more closely related to Senegal than to any of the other species used in these tests.

Attention has been called to the observation that the reagent prepared from antisera to *semitorquata* gave no more than very faint reactions with Pearlneck cells. As may be seen in table 3, the reagent prepared by the absorption of Pearlneck antiserum with the blood cells of *semitorquata* produced questionable reactions in combination with d-3 and d-7, indicating that a substance or substances related to d-3 and d-7 may be present in *semitorquata*. Otherwise there is not even a suggestion of a relationship to any other specific substance of Pearlneck.

It may be noted in table 3 that, of the nine species of Columba whose cells were used in absorbing the antiserum to Pearlneck, only those reagents prepared by the use of the corpuscles of *livia, oenas* and *picazuro* gave reactions with the cells carrying the unit substances of Pearlneck which would indicate a close relationship. For example, the absorption with the cells of both *livia* and *cenas* provided reagents which gave only questionable reactions with d-12 (columns 13 and 15). The antisera to each of these species contained antibodies to d-12, but as stated above, the relatively weak reactivity of the d-12 substance with Pearlneck antiserum will allow only the deduction that both *livia* and *oenas* have a substance related to d-12 of Pearlneck, but not necessarily identical. It is peculiar that the cells of *oenas* seemingly removed the majority of the antibodies for d-4, since the antisera to *oenas* did not contain demonstrable antibodies to d-4 (table 1). Duplicate tests of this reagent with d-4 cells gave the same results, indicating that *oenas* definitely possesses an antigenic substance capable of absorbing the majority, if not all, of the antibodies for d-4 from the Pearlneck antiserum used in these tests.

There were three species, *guinea, picazuro,* and *rufina* whose antisera (table 1) contained antibodies to the d-4 substance. However, only the cells of *picazuro* were effective in removing the major proportion, or perhaps all, of the antibodies from Pearlneck antiserum for d-4. Despite the ability of the cells of *livia, oenas* and *picazuro* to absorb the majority of the antibodies from particular Pearlneck antisera for either the d-4 or d-12 substances, or both, as described above, it seems reasonable to propose that these results should be interpreted to indicate that a similar but not necessarily identical substance to d-4 is present in *oenas* and *picazuro,* and one to d-12 is present in *livia* and *oenas.* If this interpretation is correct, it may be concluded that there are in the several species of Columba antigenic substances related to certain of the specific antigenic characters of Pearlneck, but none that have been found to be homologous.

According to the data of table 1, the antisera to the five species (Australian crested dove, Bronze wing, Brush bronze wing, Mourning dove and White wing dove) of genera other than Streptopelia and Columba contained antibodies to the d-1 substance. But the cells of none of these five species removed the antibodies from Pearlneck antiserum for the d-1 character (table 3, columns 18, 19, 20, 23 and 24). Thus substances only related to d-1 can be ascribed to these five species, but not necessarily the same substance is present in each species. On the other hand, the cells of Australian crested,

Bronze wing and Brush bronze wing, respectively, removed all the antibodies for the d-12 substance, suggesting that an antigenic character closely related to, if not identical with, d-12 is present in each of these three species.

Similarly, the antisera to the Martinique dove, Mourning dove and the White-wing dove were reactive with the d-4 character, and the cells of these species, also of the Grayson dove (*Zenaidura graysoni*) removed nearly all, or all, the antibodies from Pearlneck antiserum for the d-4 character (columns 21, 22, 23 and 24, table 3). The antiserum to the Grayson dove was not tested for its content of antibodies for the specific Pearlneck characters, but the absorption results in table 3 indicate strongly that the Grayson dove, the Martinique, the Mourning dove and the White wing dove contain antigenic substances closely related, if not identical to the d-4 of Pearlneck.

The information provided in the data of table 1 can be combined with that of table 3 to allow the general conclusion that, with but few exceptions, the antigenic substances peculiar to Pearlneck, in contrast to Ring dove, if present in other species of those tested, are present as related and not homologous substances. Of the two substances most widely distributed among these species, d-1 and d-4, only d-4 appeared to be present in homologous form, and in closely related forms, in other species. The same relationships presumably exist among the causative genes in these species.

The Distribution of the d-1 Substance of Pearlneck in Other Species

Data have been presented in the preceding pages than an antigenic substance related to the d-1 of Pearlneck is present in 15 species, other than Pearlneck, of the 25 species tested. It is pertinent, therefore, to determine the similarity or homology of this substance in each of these 15 species. To make this analysis, a dozen or more antisera to the d-1 substance were produced by immunization of rabbits with the cells of backcross birds containing only the d-1 of Pearlneck. (Only one antiserum possessed antibodies to d-1 in sufficient concentration to make it feasible for use in the tests.) The only difference between such backcross birds and Ring doves should be that the birds carrying d-1 have this substance, but otherwise their cellular antigens should be the same as those of Ring doves. An antiserum against these cells, if the antibodies to Ring dove antigens were removed by absorption with Ring dove cells, should become a reagent containing antibodies *only* to the d-1 substance. Absorptions with the cells of species reactive with this reagent would therefore provide test-fluids for testing the cells of all other species for homology or non-homology of the respective substances related to the d-1 of Pearlneck. A summary of such tests is given in table 4.

From the results of the tests of the cells of representatives of different species with the reagent for d-1, as given in column 2 and in the footnote to table 4, it may be noted that this reagent was reactive with the cells of five species of Streptopelia (*capicola, dussumieri, humilis, orientalis* and *Senegal*) besides Pearneck, and not with those of three (*Ring dove, semitorquata* and *turtur*). Although the respective antisera to five of the species of Columba (*flavirostrus, guinea, oenas, palumbus* and *rufina*) had contained antibodies to d-1 (see table 1), the antibodies in the reagent for d-1 did not agglutinate the cells of the three of these five species which were available for testing (*flaviorostris, guinea* and *rufina*). A parallel situation obtained for the lack of reaction of the cells of the Mourning dove with the d-1 reagent, whereas the antiserum against the cells of the Mourning dove contained antibodies weakly reactive with d-1 cells (table 1). It would therefore appear that this antiserum to d-1 did not contain antibodies against all

TABLE 4. *Tests for the presence or absence in various species of Columbidae of an antigenic substance similar to, or identical with, the d-1 antigen of Pearlneck*

Test cells	Agglutination of the blood cells of various species with anti-d-1 serum absorbed either by the cells of Ring Dove, or by the cells of Ring Dove plus those of each of the species as listed										
	Ring Dove[1]	capicola	dussumieri	humilis	orientalis	senegalensis	Aus. Crested	Bronze Wing	Brush Bronze Wing	megalura	White Winged
Streptopelia											
capicola	±	0	0	0	0	0	0	0	0	0	0
dussumieri	±	?	0	0	0	0	0	0	0	0	0
humilis	+	±	0	0	0	0	0	0	0	0	0
orientalis	+	±	0	0	0	0	0	0	0	0	0
Pearlneck	++										
Ring Dove	0	0	0	0	0	0	0	0	0	0	0
semitorquata	0										
senegalensis	++	+	+	+	+	0	0	0	0	0	?
turtur	0										
Australian crested	++	+	+	+	+	0	0	0	0	0	0
Bronze Wing	++	+	+	+	?	0	0	0	0	0	0
Brush Bronze Wing	+	+	+	+	?	0	0	0		0	0
Martinique	0										
Leptotila megalura	+	+	±	?	±	0	0	0	0	0	0
Mourning dove	0										
Leptotila verreauxi	++										
White Winged dove	++	±				0	0	0	0	0	0
d-1	++	+	+	+	+	0	0	0	0	0	?
Column 1	2	3	4	5	6	7	8	9	10	11	12

Symbols: see table 1.

[1] This reagent was not reactive with the cells of *Columba fasciata, flavirostris, guinea, livia, maculosa, picazuro,* or *rufina,* nor with those of the Galapagos dove (*Nesopelia galapagoensis*), Grayson dove (*Zenaidura graysoni*), Violet dove (*Leptotila jamaicensis*) or Bar-Shouldered dove (*Geopelia humeralis*).

the so-called "determinant groups," or "reactive patches" of the d-1 antigen.

In conformity with expectation from the reactions of the antisera as given in table 1, the d-1 reagent agglutinated strongly the cells of the Australian crested, the Bronze wing, the Brush bronze wing and the White wing dove. In addition, it agglutinated the cells of two species of *Leptotila, megalura* and *verrauxi,* but not of *Leptotila jamaicensis,* nor of the Martinique dove (*Zenaida aurita*), the Galapagos dove (*Nesopelia galapagoensis*), the Bar-shouldered dove (*Geopelia humeralis*) or the Grayson dove (*Zenaidura graysoni*).

Further, the analysis of the antiserum to d-1 (table 4) showed that there were antigen-antibody interactions which required the assumption of the presence of a minimum of three antibodies for explanation of the results. Thus *capicola* removed antibodies from the d-1 reagent only for itself (column 3) and the test fluid prepared by this absorption agglutinated the cells tested of all other species with cells reactive to the d-1 reagent (column 2), but with questionable reactivity with the cells of *dussumieri.* Hence the substance of *capicola* related to d-1 is only a part of d-1, and may be called d-1_a.

The respective absorptions of this antiserum to d-1 by the cells of *dussumieri, humilis* and *orientalis* removed antibodies for capicola (containing d-1_a) and for one another, but each such reagent so produced was reactive, (columns 4, 5 and 6) with d-1 cells and with those of Senegal

and other species. Since these three species removed by absorptions the antibodies for capicola, with d-1_a, each of the three species contains at least one fraction of d-1, or determinant group, in addition to d-1_a. This additional fraction may be designated as d-1_{ab}. Further, Senegal, Australian crested, Bronze wing, Brush bronze wing, *Leptotila megalura* and the White winged dove exhausted the antibodies for d-1_a (*capicola*) for d-1_{ab} (*dussumieri, humilis* and *orientalis*), as well as for one another and for d-1 itself, requiring a third fraction of d-1 in explanation of these results. This last group of species is assigned three fractions of d-1, or d-1_{abc}. However, this d-1 antiserum differed in antibody content from that of the Pearlneck antiserum analyzed in table 3, in that none of these species exhausted the Pearlneck antiserum of antibodies for the d-1 character. Therefore, as suggested earlier, it appears definite that the d-1 antiserum used in these tests did not contain all the potential antibodies to d-1. One must emphasize that explanations of the relationships which may be deduced from the results given in table 4 are limited to the specificities of the antibodies in this particular d-1 antiserum. Furthermore, from the results listed in table 3, it is clear that the d-1 substance of Pearlneck possesses at least one additional antigenic component to those of Senegal and the other species containing d-1_{abc}, so the d-1 of Pearlneck may be represented as comprising d-1_{abcd}.

Moreover, it cannot be concluded that, in the species with d-1_a, d-1_{ab} or d-1_{abc} on their corpuscles, there are no other so-called "determinant groups" associated with the substance related to d-1 which might well make for "species specificities" within each of these species. The present analysis deals only with the fractionation of antibodies against d-1 of Pearlneck, as these may be detected by presumably homologous substances corresponding to these antibodies in various other species. A pertinent example may be given of the relationship of d-1 and its related substances in Senegal, s-2.s-12 (Irwin, 1949). The d-1 character has always behaved as a unit in inheritance, while s-2 and s-12 appear to be produced by linked genes with an occasional crossover so that s-12 is available separately from the combination of s-2.s-12. It has been found that there are antigenic specificities (or determinant groups) in d-1 peculiar to Pearlneck, and also that there are specificities is s-2.s-12, particularly in s-12, which are peculiar to Senegal (Irwin, 1951). Such specificities in other species cannot be observed by the use of antisera to either Pearlneck or d-1 and can only be hypothesized, until the respective substances of the various species can be isolated in unit form as has been done for d-1 of Pearlneck, and for s-2.s-12, s-12 and probably for s-2 of Senegal.

Discussion

The experimental evidence presented in this paper has shown that certain of the antigenic characters of the blood cells which distinguish one species (Pearlneck) from another (Ring dove) may be found as related substances, rarely indeed as homologous substances, in other species of Columbidae. Presumably, the relationships of the antigenic substances as described also reveal the same or nearly the same sort of relationships among the genes producing these respective substances in the various species.

In addition to the antigenic substances, and therefore their causative genes, which have been considered, there are those substances which are held in common among these related species. Buchbinder (1934) has described an antigen which in presumably homologous form is present in various species of many orders of the class Aves, including the Columbiformes, within which are placed the species of this study. This antigen is related to one which occurs in some strains of Pasturella. There undoubtedly are other antigenic substances than this particular antigen, which was detected by virtue of its heterophilic nature, which are held in

common by all these species. In fact, it would be expected that there would be many more cellular and serum antigens which are *shared* by the various species of this study than there are which *differentiate* one of these species from another. Furthermore, the greater the proportion of antigens—therefore their causative genes—shared by any two species to those species-specific to each species, the closer will be the relationship between the species. This statement is a slight variation from one made previously by Morgan, Bridges and Sturtevant (1925) that "Related species must have many genes in common—they are in fact similar only because of this group of common genes."

The majority of the studies on homologous genes in various species have compared the end products of respective gene mutations which have occurred within related species. Pertinent examples and references to source materials are given in papers by Haldane (1927), Harland (1936), Morgan, Bridges, and Sturtevant (1925), Muller (1940), Silow (1941), Spencer (1949), and Sturtevant and Novitski (1941), to cite only a few of the many papers on this subject. A critical treatise on the evolution and function of genes has recently appeared (Sturtevant, 1948). Evidence of a somewhat different kind suggesting the homology of certain genes which are concerned with the development of eye color in three orders of insects has been presented by Beadle, Anderson and Maxwell (1938). And Landsteiner and Miller (1925) have shown that the A and B antigens of human red blood cells, whenever present in chimpanzees, gibbons and the orang, were indistinguishable from those of humans and therefore presumably homologous, implying homology of the causative genes. All these examples may be taken as indicative of the stability of the respective causative genes during the evolution of the various species.

The experiments reported in this paper have been primarily concerned with determining the presence or absence in related species of antigenic characters of the blood cells which differentiate one species (Pearlneck) from another (Ring dove). A related species, Senegal, has been found (1) to possess nearly all the antigenic substances which are common to both Pearlneck and Ring dove, and (2) a part or all of each of nine cellular antigens which distinguish Pearlneck from Ring dove (Irwin, 1949). Four of these nine (d-3, d-4, d-5 and d-12) are indistinguishable and therefore seemingly identical in Pearlneck and Senegal, implying homology of the respective causative genes in the two species—homologous genes in these two species which produce species-specific differences in comparison with another species (Ring dove). The remaining five antigenic substances (d-1, d-2, d-6, d-7 and d-11) of Pearlneck have related but not identical substances in Senegal, implying that the causative genes are not homologous. It is obvious that, in the evolution of these three species, changes have occurred in the gene or genes effecting the nine cellular antigens distinguishing Pearlneck from Ring dove, and in the five which differentiate Pearlneck from Senegal.

A fractionation of the antigenic character d-4 of Pearlneck and its homologue s-6 of Senegal has been noted, which can most reasonably be explained as resulting from a crossover of linked genes. A similar fractionation of another antigen of Senegal, s-2.s12, related to the d-1 of Pearlneck, has been observed, also undoubtedly following a crossover. The possibility therefore exists that each of the nine species specific antigens of Pearlneck, as contrasted with Ring dove, may be the result of the joint action of two or more linked genes, and that crossingover has not yet been observed, except as stated.

The results cited in the preceding pages allow the general conclusion that, with but one exception (in *semitorquata*), each of the 23 species tested, other than Pearlneck and Ring Dove, contained one or more antigenic substances at least re-

lated to those specific to Pearlneck, in contrast to Ring dove. Furthermore, with but few exceptions apart from the cellular antigens of Senegal as described —exceptions in which the d-4 antigenic substance was primarily involved—there was only similarity, not homology, in any of these antigenic substances in any other species to that of Pearlneck (data of table 1 and table 2).

As an example, the d-1 antigen was one of the most cosmopolitan of those specific to Pearlneck, since it or a related substance was present in 16 of the 25 species tested (including Pearlneck), and was absent in nine of the 25 (including Ring dove). The observations also showed that in each of these 15 species other than Pearlneck, the substance was antigenically related to the d-1 of Pearlneck, but not homologous in any species to the d-1.

The evidence presented in table 4 shows that there are different so-called fractions or specificities of the d-1 substance in several species; that called d-1_a *capicola;* d-1_{ab} in *dussumieri, humilis* and *orientalis;* d-1_{abc} in Senegal, Australian crested, Bronze wing, Brush bronze wing, *Leptotila megalura* and the White winged dove; and at least one additional fraction or specificity in Pearlneck, making it d-1_{abcd}. These represent the respective specificities of the d-1 substance of Pearlneck which are found in various species, as revealed by the antibodies in a single antiserum to the d-1 substance, and indicate the extent to which changes in the respective causative genes must have occurred during the evolution of these species. It is possible that further complexities may be found in other antisera to d-1 in the relationships among these same species of the antigenic fractions of the d-1 substance, but they will be superimposed on those listed above.

It may be assumed that an ancestral form to all these species contained a cellular antigen from which must have evolved the various forms of the d-1 substance, as they now exist in the several species with related substances. One can only guess whether the d-1-like substance in this hypothetical ancestral species was similar to or totally unlike the present d-1 antigen, *i.e.,* whether the changes in the genes in the various species have been in the direction of making for similarities to, or differences from, the d-1 substance. The six species (Senegal, Australian crested, Bronze wing, Brush bronze wing, *Leptotila megalura* and White winged dove), which show the greatest resemblance to the d-1 antigen, are widely separated in their native habitats—the Senegal is found in Africa, the Australian crested, Bronze wing and Brush bronze wing in Australia, *Leptotila megalura* in South America, and the White winged dove in southwestern United States and Mexico. As these different species evolved, are the chances greater that the gene or genes effecting the cellular antigens related to d-1 mutated toward the same specificities (d-1_{abc} in the six species) or in the opposite direction? It seems somewhat more reasonable to assume that the gene mutations in the various species made for antigenic specificities away from those of the ancestral form of d-1, rather than otherwise. If this be the case, the six species with the d-1_{abc} specificity are those which, despite their geographical diversity, have undergone less genetic change with respect to the d-1 antigen than have those with less resemblance to the d-1_{abcd} of Pearlneck. Within the three species (*dussumieri* and *humilis* from the Philippine Islands and surrounding territory, and *orientalis* from eastern Asia) with the d-1_{ab} fraction, there presumably was a greater genetic change than in the six species with d-1_{abc}, while the greatest change from the ancestral form occurred in *capicola,* which is characterized as possessing only d-1_a.

If only one gene is involved in each of these species in the production of d-1 and its related antigenic substances, its different forms in the various species might conceivably be explained by assuming that the causative genes in the various species

form an allelic series. Under such an explanation the respective genes for the antigens related to d-1 in the various species would have relationships to each other paralleling those existing for certain of the so-called blood groups in humans (Race, 1950; Wiener and Wexler, 1952), in cattle (Stermont, Owen and Irwin, 1951), and in chickens (Briles, McGibbon and Irwin, 1950).

However, since the substance (s-2.s-12) in Senegal related to d-1 has been shown (*loc. cit.*) to be divisible into two components, presumably as a result of the separation of linked genes following crossing over, it is an open question as to whether d-1 and other d-1-like substances in the different species are the products of single genes. If two or more linked genes produce the d-1 and d-1-like substances in the various species, one could hypothesize that one or more of the linked genes produced the antigenic substances common to various species—a gene $d\text{-}1_a$ in *capicola;* genes $d\text{-}1_a$ and $d\text{-}1_b$ in *dussumieri, humilis* and *orientalis;* genes $d\text{-}1_a$, $d\text{-}1_b$ and $d\text{-}1_c$ in Senegal and the five other species; and in Pearlneck genes $d\text{-}1_a$, $d\text{-}1_b$, $d\text{-}1_c$, and at least one other gene $d\text{-}1_d$. Any species-specific effects associated with the d-1 substance in these species would then be expected to be produced by genes linked to those listed. A combination of these two alternative explanations for the relationships among the various species would be at least equally plausible to either one alone.

It may be assumed that in Ring dove there is a contrasting antigenic substance to the d-1 Pearlneck, and that this may be demonstrated by the same kind of technics that was used to detect in *Columba livia* the contrasting substance C′ to the C of *C. guinea* (Miller and Bryan, 1951). A basic question then is whether the causative gene or genes of d-1 in Pearlneck and its contrasting substance in Ring dove, bear the same relationship to each other as do contrasting alleles within a species. If so, considerable antigenic similarity should exist between the d-1 of Pearlneck and its contrasting substance in Ring dove, on the premise that alleles effect more or less slight differences in their end products, rather than changing entirely the specificity of the end product. It would also be anticipated that those species, of the 25 tested, which did not not possess on their blood cells an antigenic substance related to the d-1 of Pearlneck, would then carry a substance at least related to the substance in Ring dove contrasted to the d-1 of Pearlneck. Whether this expected result will be realized can be determined only by further tests.

The relationships among the various species of the antigenic substances peculiar to Pearlneck, other than the d-1 antigenic character, most probably follow the same kind of pattern that obtains for the d-1 and its related substances. Certain of these relationships will be discussed in subsequent papers.

Acknowledgments

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Summary and Conclusions

It seems reasonable to conclude that the proportion of antigenic substances which are *shared* by any two related species—as by Pearlneck and Ring dove—is greater than that which distinguishes either from the other. The number of such substances cannot be determined, since these substances common to the species do not separate in the offspring of their species hybrids. On the assumption that the antigenic substances *common* to

two related species are gene-determined, such substances would be the products of homologous genes in the two species. The products of alleles in either species with contrasting effects might or might not be present in the other species.

Nine antigenic characters of the red blood corpuscles which distinguish Pearlneck (*St. chinensis*) from Ring dove (*St. risoria*) have been used as distinct "tester substances" to determine the presence or absence of each antigenic substance in the blood cells of 23 other species of Columbidae. Only rarely have any of these nine antigens been found as indistinguishable and presumably identical antigenic substances in these other species. Usually, if a particular substance of Pearlneck was demonstrable in another species, it was present in that species as a related substance.

Only one species tested (Senegal, *St. senegalensis*) contained antigenic substances either related to or identical with each of these nine antigenic characters which distinguish Pearlneck from Ring dove. The other species possessed one or more of these antigenic substances of Pearlneck, and the great majority of these were contained as related antigens to those of Pearlneck. One such antigenic substance of Pearlneck, the d-1 antigen, was present in 10 other species than Pearlneck in three different but related forms.

For those antigenic substances which in another species are indistinguishable from and presumably identical with a particular substance in Pearlneck, it may be assumed that the causative genes in the two species are homologous. And for those cellular antigens which in another species are related to but not identical with an antigen of Pearlneck, it is assumed that the causative genes are likewise related but not homologous.

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THE ROLE OF HYBRIDIZATION IN THE EVOLUTION OF THE LEAFY-STEMMED GILIAS

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INTRODUCTION

The Leafy-stemmed Gilias, a group of annual herbs occurring in great diversity on the Pacific slope of North America, have long constituted a difficult taxonomic problem. They comprise one of those so-called "critical groups," in which the traditional methods of taxonomy have failed to elucidate the divisions between the species. It is not possible with the aid of existing monographs and floras to properly identify more than a fraction of the diverse forms of Leafy-stemmed Gilia that occur in nature. A taxogenetic study of the group was undertaken some years ago for the purpose of discovering the evolutionary causes responsible for this situation. The basic species were first delimited and described (Grant, 1950 to 1953, hereafter cited simply as parts I to V of a series). The genetic relationships between the species were next investigated; part VI contains descriptions of the external morphology, cytology and fertility of artificial hybrids between the different species. The documentary evidence is set forth in this and preceding papers of the series. The purpose of the present paper is to offer an explanation of the characteristic pattern of evolution of the group.

GENERAL DESCRIPTION

The Leafy-stemmed Gilias comprise a natural subdivision of the genus *Gilia*, family Polemoniaceae, technically known as the section *Eugilia*. The plants composing this section are herbaceous annuals with blue-violet flowers borne in loose cymes or in capitate heads. The stems are more or less leafy throughout their length, in distinction to some related sections which consist of rosette plants. The members of the group grow on loose sandy soils in the more open communities of the Pacific slope of North America; there is a secondary center of distribution in temperate South America. The nine or ten species of Leafy-stemmed Gilia fall into three main assemblages. The following brief recapitulation, together with the illustrations in figures 1 and 2 and the distribution maps of figure 3, will acquaint the reader with the general characteristics of the plants.

The Gilia tricolor group. These are small plants which often form very extensive populations on the rolling foothills and plains of California behind the coastline. The inflorescence is a loose cyme and the corolla is campanulate in form and often tri-colored due to the presence of an orange tube and five pairs of oval purple spots against the blue-violet background of the lobes. The plants are self-compatible, so that pollination of the flowers by bees brings about a combination of selfing and outcrossing. There are two species, *G. tricolor* Benth. (with two subspecies) and *G. angelensis* V. Grant, both of which are diploid with 9 pairs of chromosomes.

The Gilia capitata group. These are plants of larger size with the flowers borne in heads. The corolla is funnelform and blue-violet throughout. The two species, *G. capitata* Sims and *G. achilleaefolia* Benth., usually occur in disjunct local colonies in the hills and mountains of the Pacific states. *Gilia achilleaefolia* is self-compatible and includes, among its numerous variations, some large-flowered forms which are partially cross-pollinated by bees and partially self-pollinating, as well as small-flowered forms which are automatically self-polli-

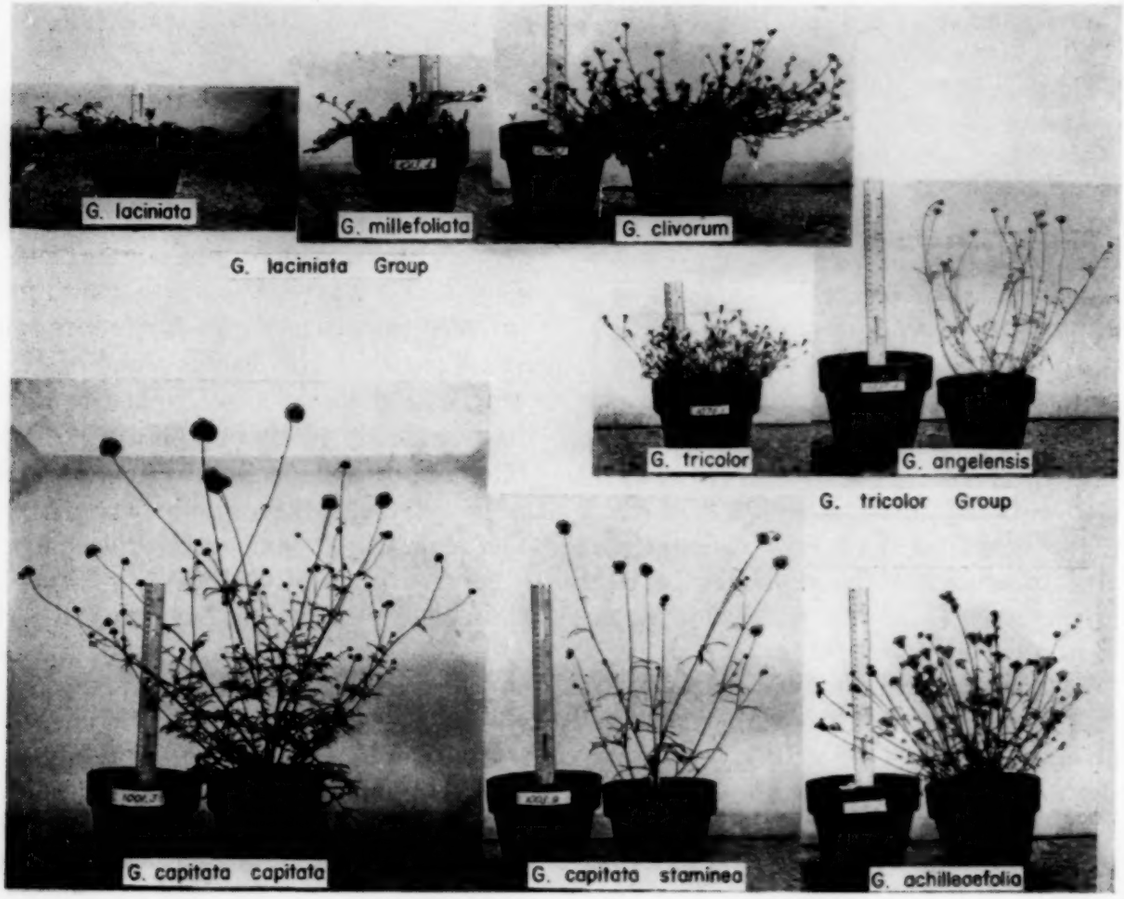

FIG. 1. Growth habit of eight kinds of Leafy-stemmed Gilia, grown under uniform conditions.

nated. *Gilia capitata* contains both self-compatible and self-incompatible races, all of which depend upon insects, mainly bees, for pollination. The eight named subspecies of *G. capitata* fall into three main series: a group of races which occur in mountainous habitats from central California to British Columbia (*G. c. capitata,* etc.); a group of races which inhabit sandy plains and dunes in the San Joaquin Valley and along the Pacific strand (*G. c. staminea,* etc.); and forms in the mountains and foothills of southern California and northern Baja California (*G. c. abrotanifolia*). Both *Gilia capitata* and *G. achilleaefolia* are diploid with $n = 9$.

The Gilia laciniata group. These are maritime plants, often with a prostrate habit of growth and very glandular herbage, which usually grow near the Pacific Ocean. Several of the species are self-compatible and automatically self-pollinated. In North America the group is represented by three species: *G. millifoliata* Fisch. et Mey., a diploid species which grows on coastal sand dunes; *G. clivorum* V. Grant, a tetraploid occurring along the coast and in the interior Coast Ranges of central California; and *G. nevinii* Gray, an endemic on three islands off the coast of southern California and Baja California. The group is represented in South America by two or three species of tetraploids and diploids which, for the time being, may all be referred to as *Gilia laciniata* Ruiz et Pavon sens. lat.

THE VARIATION PATTERN

The species of Leafy-stemmed Gilia are separated in nature by ecological differences, by differences in the period of flowering, and by differences in the form, color pattern and odor of the flowers

which enable the pollinating bees to discriminate between them. The reproductive isolation is continued internally by strong incompatibility barriers imposed at various stages in the processes of fertilization and seed formation. It is completed by several kinds of hybrid sterility and by the inviability of a part of the later generation progeny of the hybrids. The species of Leafy-stemmed Gilia are, in short, very well isolated (see part VI).

Knowing these facts, one might have predicted that the Leafy-stemmed Gilias would group themselves into an array of distinct species. Yet as we have seen in the introduction this is by no means the case. A satisfactory classification of the group has hitherto eluded the best efforts of taxonomists; it is difficult, perhaps impossible, to devise a key for the certain identification of all the various forms of Leafy-stemmed Gilia even now. The reason for this situation may be sought in a peculiarity of the variation pattern of the group. The species, as delimited on the basis of crossability and hybrid sterility, are all more or less polytypic and include various intergrading local races. Some of the branches of a species then seem to intergrade with certain branches of other species.

Gilia capitata, with its globose flowering heads, approaches *G. millefoliata* with its few-flowered glomerules, on Cape Mendocino in northern California, where colonies exist with loose heads composed of an intermediate number of flowers on long pedicels. They are evidently members of *G. capitata* which vary in the direction of *G. millefoliata* without completely bridging the gap between the two species.

The resemblances in general habit and floral characters between the more southerly races of *G. capitata* and the large-flowered capitate races of *G. achilleaefolia*

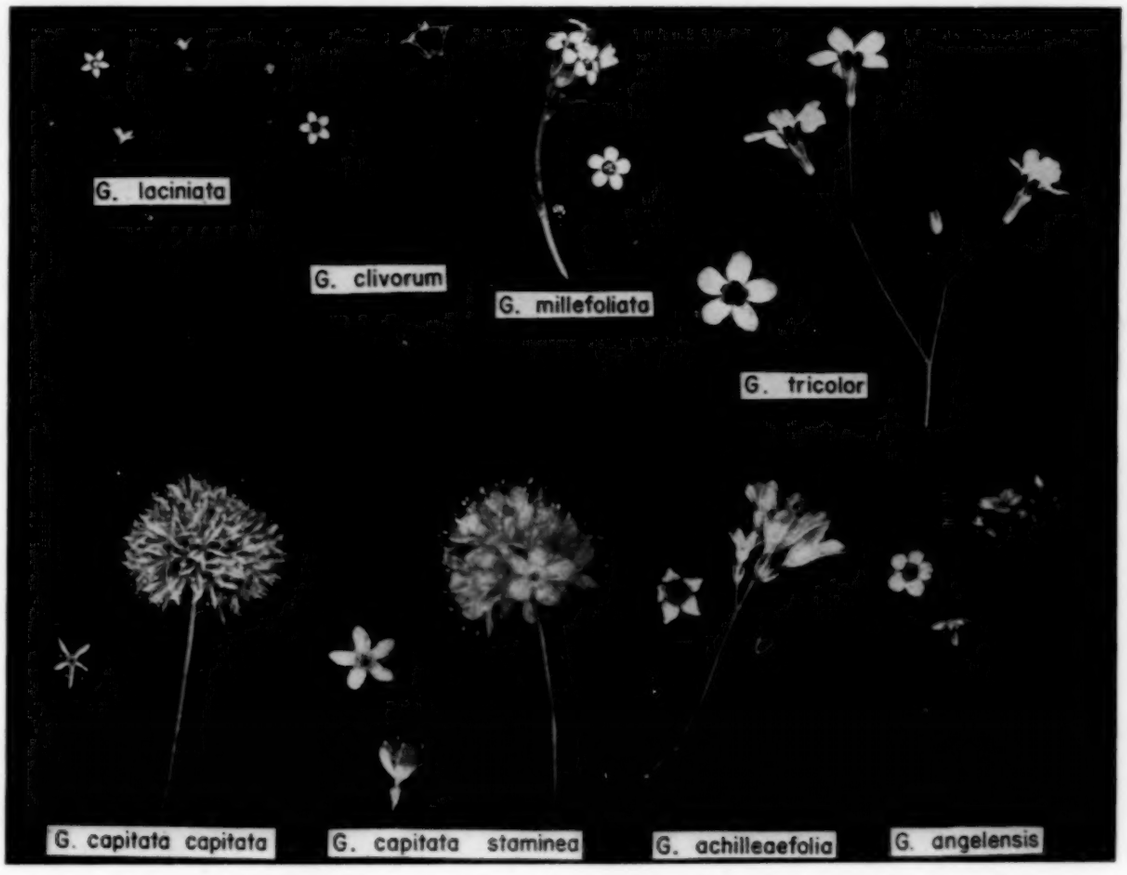

FIG. 2. Inflorescence and flowers of eight kinds of Leafy-stemmed Gilia. From the same plants as in figure 1. All to same scale.

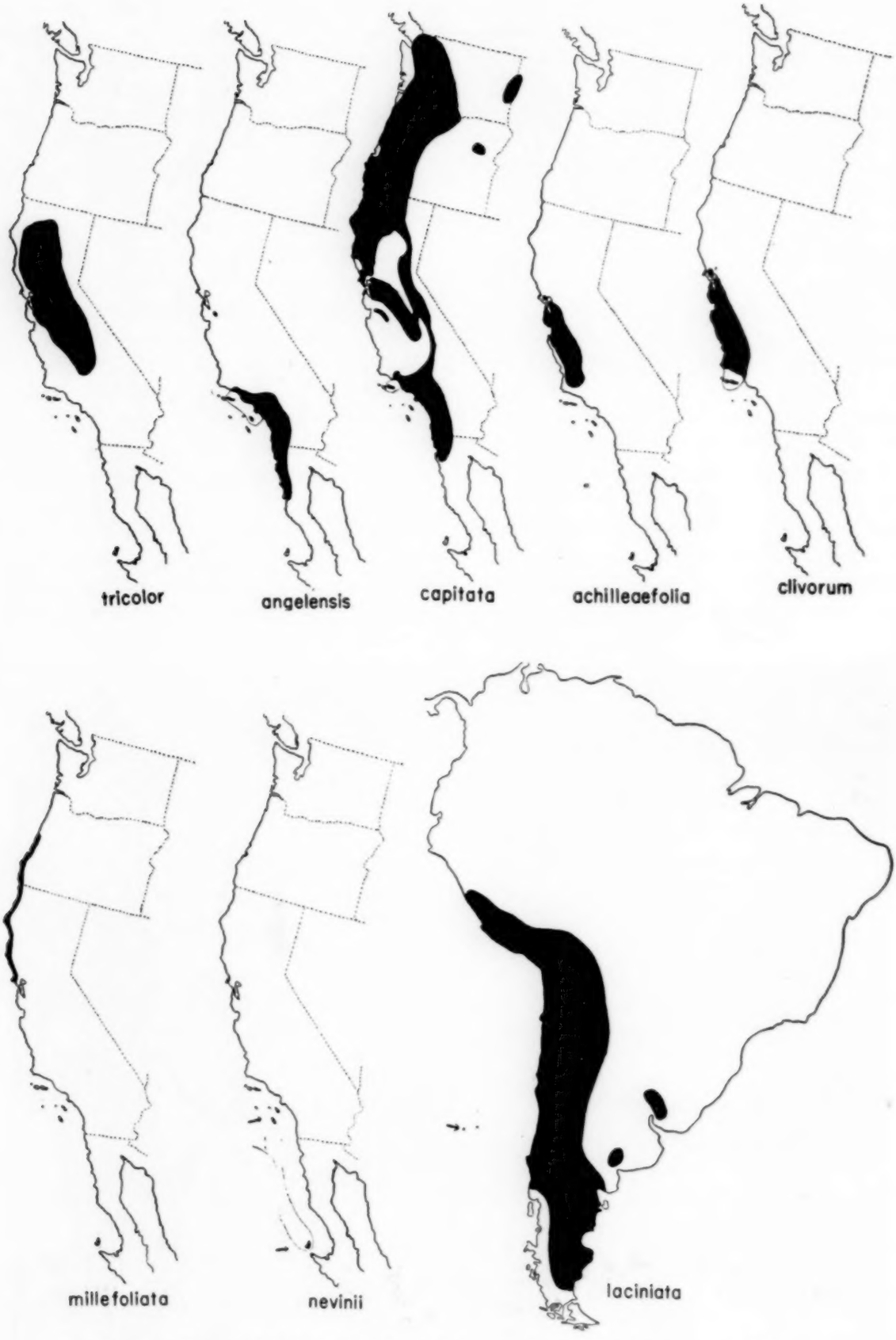

FIG. 3. Geographical distribution of the species of Leafy-stemmed Gilia.

are so striking that the two species have been united in most taxonomic treatments proposed during the past century. The large-flowered and capitate races of *G. achilleaefolia* then intergrade into small-flowered and cymose races of the same species which are so dissimilar that the two types have been maintained in separate species for over a century.

The trailing, small-flowered races of *G. achilleaefolia* can only be distinguished with difficulty from the trailing, small-flowered types of *G. clivorum,* while the latter intergrade with larger-flowered, purple-spotted, semi-succulent forms of *G. clivorum* which closely resemble *G. millefoliata.* This particular situation is reflected in the earlier taxonomic treatments of the genus, wherein the populations of *G. clivorum* have not been recognized as constituting a distinct species, but have been assigned partly to *G. millefoliata* and partly to the small-flowered types of *G. achilleaefolia.*

The small-flowered, pale-colored, cymose races of *G. achilleaefolia* are so similar in aspect to *G. angelensis* that these two species have not hitherto been separated in taxonomic treatments. Forms of *G. angelensis* are also occasionally found which, in their dense, more capitate inflorescences and more erect habit of growth, vary towards *G. capitata.* This tendency of some populations of *G. angelensis* is matched by a counter-tendency of some types of *G. capitata* in the same area to develop loose heads with fewer flowers and longer pedicels than usual.

To summarize, partial or complete intergradation connects the species of Leafy-stemmed Gilia as follows:

(i) *millefoliata* ↔ *capitata* ↔ *achilleaefolia* ↔ *clivorum* ↔ *millefoliata.*

(ii) *capitata* ↔ *achilleaefolia* ← *angelensis* ↔ *capitata.*

Figure 4 shows what happens when we plot the variations of one species, *G. achilleaefolia,* together with those of the geographically and morphologically closest branches of two other species, *G. capitata* and *G. angelensis,* for two important diagnostic characters. A broad overlap is apparent in the variation patterns of the different species. Similar results have been obtained with other characters and other combinations of species.

If intergradation were to be used as a criterion of subspecific distinction, and if the limits of species were to be defined by morphological discontinuities, as is customary in taxonomic practice, the Leafy-stemmed Gilias of continental North America might logically be grouped into just two species, *G. tricolor,* and a huge and complicated assemblage composed of all the other forms. A little field work would soon bring this disposition into conflict with another established criterion of systematics, which holds that two or more natural populations coexisting in one territory are necessarily the members of distinct species, for there are innumerable sympatric contacts between the various kinds of Leafy-stemmed Gilia (see part VI). The foregoing paradox is one which actually confronted the writer at an early stage of the investigations. The genetic evidence, when it became available, was decisive and confirmed the evidence of geographical distribution, rather than that of the variation pattern. There can no longer be any doubt that the vast and complex assemblage of Leafy-stemmed Gilias in California is actually composed of several well isolated species.

Evidences of Hybridization

An explanation is needed for the presence of the intermediate forms which obscure the morphological distinctions between the well isolated species. It is believed that the intermediate types owe their existence to occasional natural hybridization between the species. This hypothesis is supported by several lines of evidence.

In the first place, there is morphological evidence of introgressive hybridization between some of the species, as for example between *G. capitata pacifica* and *G. millefoliata* on Cape Mendocino; between *G. capitata abrotanifolia* and *G.*

angelensis in Santa Ana Canyon in the Santa Ana Mountains; and between *G. capitata staminea* and *G. achilleaefolia* in the Inner South Coast Range near Coalinga. In each case a population deviates in its morphological characters from the norm of its species and approaches the conditions found in some other species with which it is growing. The characteristics of the plants are such as to suggest

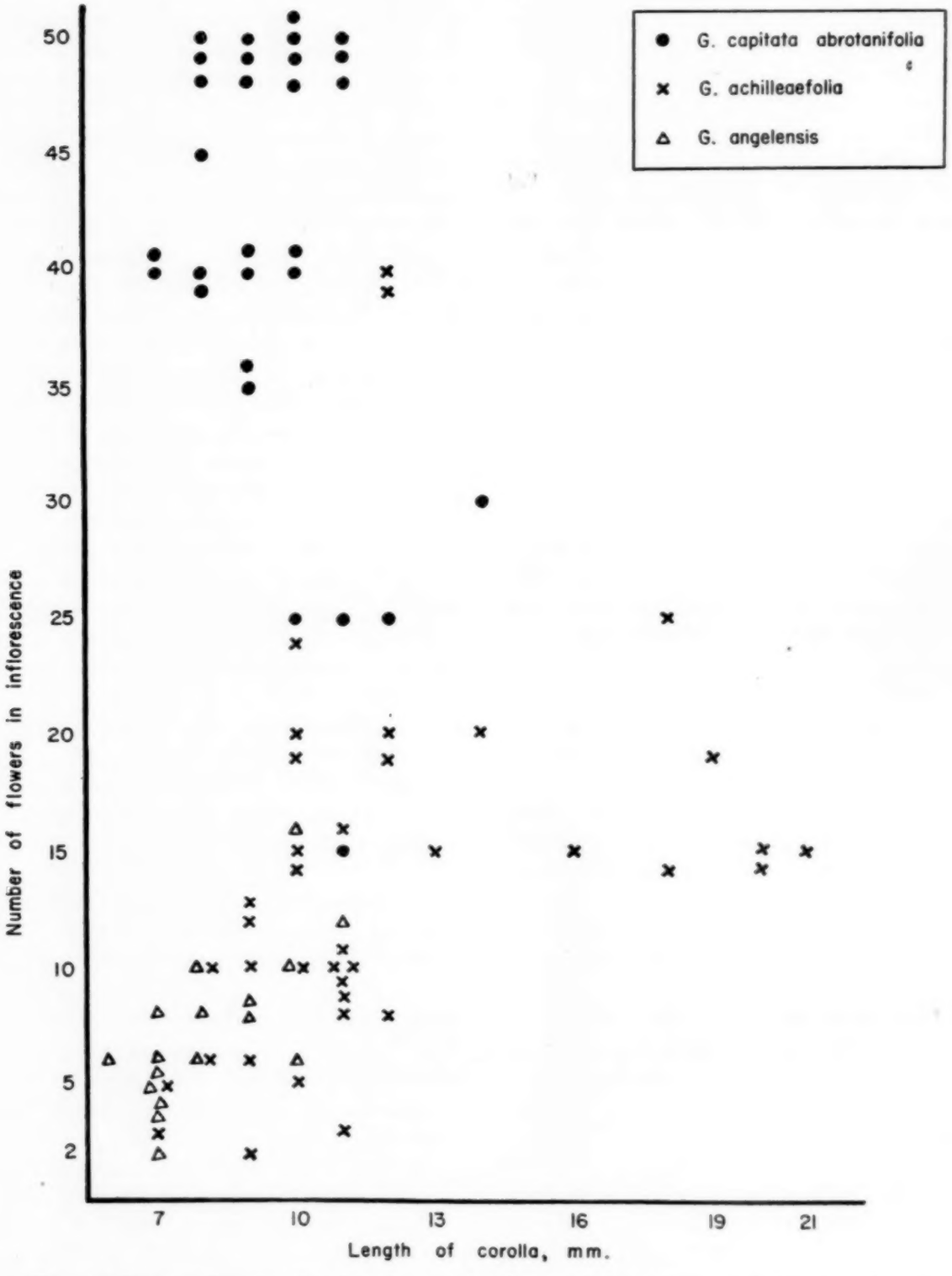

FIG. 4. Scatter diagram showing the overlapping variation patterns of three species. Each symbol represents one population.

initial crossing followed by backcrossing to one or the other parental species. The deviating populations are fully fertile in the wild and their progeny, in those cases where they have been grown, are fertile also in the experimental garden. The fertility relationships of the deviating types with the parental species have not yet been investigated. Population samples and progeny tests show that they are not excessively variable. The populations are thus probably derivatives of introgressive hybridization, which through the years have become genetically stabilized.

The introgressive hybrids show that natural hybridization does occur. The tetraploid species, *G. clivorum,* bears witness to the fact that the natural hybrids may sometimes become established as important biological entities. This species is a product of hybridization between *G. millefoliata* and *G. achilleaefolia.* It crosses with the artificial allotetraploid *G. millefoliata-achilleaefolia* to produce a fertile first generation hybrid with normal meiosis (part V).

It is significant that the taxonomic confusion in the Leafy-stemmed Gilias is confined to just those species which the crossing experiments show will occasionally hybridize (part VI). *Gilia tricolor,* which apparently forms a comparium by itself, since it is not known to hybridize with any other species either in nature or in the breeding plot (part III), presents no difficulties at all from the taxonomic point of view.

A comparison of the different species with regard to their inter-colonial variability is enlightening. The hereditary variability from population to population is very great in some of the species, whereas in others it is much less. The entities which occupy the most extensive and diverse territories, and which therefore might be expected to include ecotypes adapted to the greatest number of climates are *G. capitata capitata* and *G. tricolor.* Another wide ranging taxon is *G. capitata abrotanifolia.* Each of the two subspecies of *G. capitata* occurs very commonly in several mountain ranges; *G. tricolor* occurs very widely on the foothills of two large mountain systems and in several intermontane valleys. These entities are largely, and in some cases entirely, cross-pollinated. Yet they are far from being the most variable species or races.

Gilia capitata pacifica, which occupies a narrow strip along the coast of northern California, possesses a great deal more inter-colonial variability than does *G. c. capitata* in the whole area from San Francisco Bay to Vancouver Island and from the maritime Coast Ranges to Idaho and eastern Oregon. It is more variable than *G. millefoliata,* which is equally widely diffused in the same area. *Gilia achilleaefolia,* which is restricted to a relatively small area in the South Coast Range and which reproduces partly or entirely by self-pollination, is easily the most variable species in the whole section. *Gilia clivorum,* with a more extensive area of distribution but capable of even less cross-breeding, is likewise highly variable. The wide-ranging and extensively cross-breeding *Gilia tricolor,* which occurs in the territory of *G. achilleaefolia* and *G. clivorum* and elsewhere too, cannot begin to compare with these two species in variability.

It is doubtful whether the excessive variability of *G. capitata pacifica, G. achilleaefolia* or *G. clivorum* can be entirely explained as a result of the accumulation of genetic changes which have occurred within these species or subspecies themselves. A far more likely explanation, in the opinion of the author, is that the variability in excess of the amount present in *G. capitata capitata* and *G. tricolor,* is a consequence of hybridization between reproductively isolated populations.

It is noteworthy in this connection that the most variable entities are also intermediate in a large number of morphological and ecological characters between certain other entities. *Gilia capitata pacifica*

is intermediate between *G. capitata capitata* and *G. c. chamissonis* (part I); *G. achilleaefolia* is intermediate between *G. tricolor* and *G. c. staminea* (part IV); *G. clivorum* is intermediate between *G. millefoliata* and *G. achilleaefolia* (part V). The association of morphological intermediacy with great genetic variability is a known result of interracial or interspecific hybridization. It has therefore been suggested in earlier papers that the variable and intermediate types are derivatives of hybridization.

The Original Species

An obvious question concerns the identity of the original species between which the hybridizations occurred. The author regards the original species in North America as being: *G. tricolor* and *angelensis* in the *tricolor* group; *millefoliata* and *nevinii* in the *laciniata* group; and *abrotanifolia, capitata* and *staminea* in the *capitata* group.

The circumstance that the last named entities are treated taxonomically as subspecies should not be allowed to obscure the biological facts, which point to the occurrence of speciation in their past history (parts I and II). *Gilia capitata staminea* grows in the territory of *G. c. capitata* today in the Vaca Mts. of central California. Gene exchange between them is prevented by the fact that the former type inhabits the sandy washes and blooms early, while the latter grows on rocky canyon slopes and flowers later. Colonies of a maritime ecotype of *staminea* (taxonomically, *G. c. chamissonis*) and a maritime ecotype of *capitata* (*G. c. tomentosa*) occur less than 500 feet apart at Tomales Bay. This distance is within the range of seed dispersal of Gilias. Yet the sympatric populations do not hybridize today. This is shown by the fact that the *capitata* populations are 100% pure in respect to a recessive marker gene, controlling an easily scored pubescence character, the dominant allele of which is carried by the neighboring *staminea* populations. The isolated populations are separated in this case by pronounced ecological differences accompanied by weak incompatibility barriers. A foothill ecotype of *staminea* (*G. c. pedemontana*), finally, grows sympatrically with *G. c. abrotanifolia* in the Sierra Nevada, where gene exchange is barred by ecological and ethological isolation.

The existence of reproductive isolation between *G. c. capitata, G. c. staminea* and *G. c. abrotanifolia* shows that these entities have reached the stage of divergence characteristic of species. The process of speciation was reversed, however, by subsequent hybridization between *G. c. staminea* and *G. c. capitata,* in consequence of which a series of intermediate and highly variable populations arose in certain ecologically intermediate areas (part I).

The author's conception of phylogeny in the North American Leafy-stemmed Gilias is portrayed in figure 5. The skeletal phylogenetic tree in this figure, which is outlined in solid black lines, depicts the divergence of the original species just enumerated. Subsequent hybridizations between these basic species, indicated in the figure by the broken lines, have resulted in the origin of additional colonies, races and species intermediate between the former. The hybridizations have converted the phylogenetic tree into a phylogenetic net.

It is improbable that figure 5 does full justice to the actual complexity of the situation. For example, hybridization may have occurred in the remote past between *G. tricolor* and *G. millefoliata* when the former species did not constitute a separate comparium. Such hybridization would explain the presence in *G. millefoliata* of purple corolla spots identical in number and pattern, but a shade less bright in color, to those found in *G. tricolor.* Since the corolla spots are polygenically determined, as shown by the occurrence of at least four phenotypic classes of brightness in the various species and hybrids, the likelihood of this character having arisen independently in

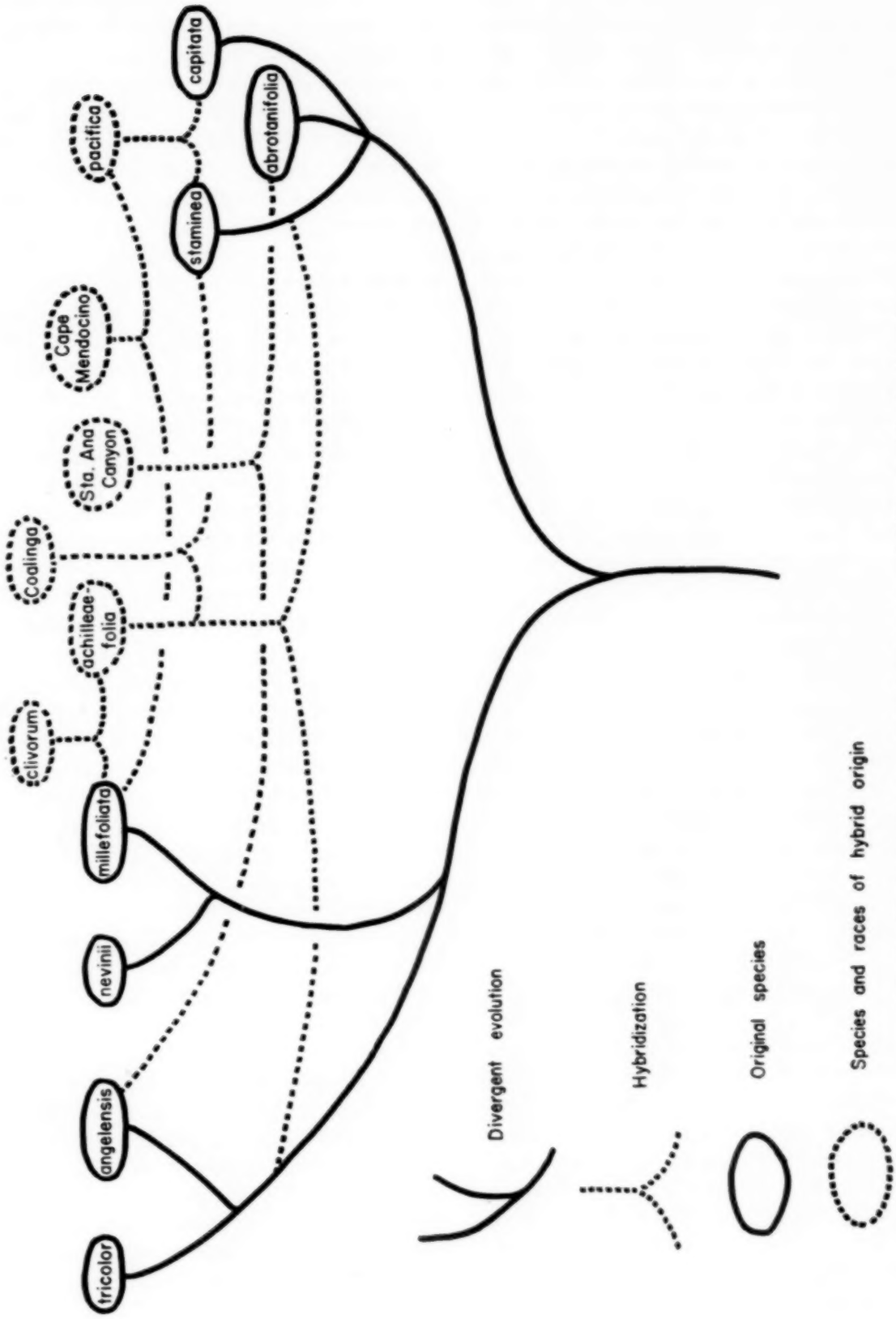

FIG. 5. Phylogenetic net of the Leafy-stemmed Gilias in North America.

two different evolutionary lines is less than the chance of its having been introduced into one of the lines by hybridization. The hypothesis of a hybrid origin of the corolla spots in *G. millefoliata,* which occupies an area contiguous to *G. tricolor,* would also explain the absence of spots in the closest relatives of *G. millefoliata,* namely *G. nevinii* and *G. laciniata,* which occur far to the south of the range of *G. tricolor* and may have never come into contact with this species.

The available evidence suggests that the pattern of hybridization was established in the Leafy-stemmed Gilias in ancient times. The age of the tetraploid *G. clivorum* is sufficiently great to have enabled it to fill a niche of its own in the oak woodland and grassland savannah of the California Coast Ranges over a longitudinal distance of 400 miles. Consideration of the amount of time required for the colonization of an area of this size imposes a definite minimum limit on the antiquity of this species. Yet *G. clivorum* could not have arisen until one of its diploid ancesters, *G. millefoliata,* had acquired the characteristic corolla spots from *G. tricolor* by introgression and until the other diploid ancestor, *G. achilleaefolia,* had originated following hybridizations between *G. tricolor* and *G. capitata.* If *G. clivorum* is old, the hybridizations of its ancestral diploid species with *G. tricolor* must be even older.

The Hybrid Complex

The Leafy-stemmed Gilias resemble in their variation pattern some other taxonomically difficult groups, such as *Rubus* § *Eubatus,* the *Crepis occidentalis* group, *Phleum pratense* and its allies, the *Artemisia tridentata* group, *Oenothera* § *Euoenothera,* and the like, in that the species are not well separated morphologically. The resemblance was puzzling at first, because the species of Leafy-stemmed Gilia are sexual, possess a normal chromosome cycle, and are predominantly diploid. They evidently do not comprise a polyploid complex, an agamic complex, or an assemblage of ring-forming structural heterozygotes. There is, however, an evolutionary factor common to all these complexes, which may explain their resemblances on the taxonomic level. That factor is hybridization. In some groups it has been associated with numerical or structural changes of the chromosomes or with changes in the method of reproduction. The effects of interspecific hybridization, however, are not necessarily manifested in easily recognized permanent alterations of the chromosomal mechanism. Hybridization may blur the distinctions between species without producing any easily recognizable cytogenetic features in the hybrid derivatives.

Groups of species in which hybridization has obscured the morphological discontinuities between the basic diploid types may be termed collectively *hybrid complexes.* The known types of hybrid complexes, arranged in order of decreasing taxonomic complexity, are as follows:

Agamic complex (Babcock and Stebbins, 1938), in which the hybrids or hybrid derivatives reproduce partially or wholly by unfertilized seeds or bulbils, which may attain a wide dispersal. Typical agamic complexes are *Crepis* § *Psilochaenia* (*op. cit.*) and *Rubus* § *Eubatus* (Gustafsson, 1942).

Polyploid complex (Babcock and Stebbins, 1938), in which the derivatives of hybridization are sexual polyploids. This type of hybrid complex is found in the genera *Clarkia* (H. Lewis, unpublished), *Iris* (Viosca, 1935; Anderson, 1936; Lenz, unpublished), and *Artemisia* (Keck, 1946; Ward, 1951).

Heterogamic complex, in which the products of hybridization are permanent structural heterozygotes. The best example of a heterogamic complex is *Oenothera* § *Euoenothera* (Cleland *et al.,* 1950).

Clonal complex, in which the hybrids reproduce mainly or entirely by clonal divisions and therefore tend to remain

more or less localized. A good example is afforded by *Opuntia.*

Homogamic complex, in which the hybrid derivatives are sexual with a normal meiotic cycle and are predominantly or entirely diploid. Structural hybridity may or may not be present in the first generation hybrids; if present, it is eliminated in the later generation progeny of the hybrids by selection for sexual fertility. Homogamic complexes are developed in *Quercus* (Stebbins, 1950, chap. 2; Tucker, 1952a, 1952b; Benson, 1951), *Ceanothus* (McMinn, 1944; Nobs, 1951), *Aquilegia* (Grant, 1952a), and *Gilia* § *Eugilia.*

In their historical development, agamic, polyploid, heterogamic and clonal complexes apparently pass through a period of expansion and a period of decline. The complexes in which sexual recombination is inhibited in one way or another can expand until they have reached a climax of variability. Their fate from this point on is to gradually die out; they can give rise to no new lines of evolution (Stebbins and Ellerton, 1939; Stebbins, 1940, 1941, see also 1950, chaps. 8–11). In the homogamic complex, on the other hand, the derived forms are as fully capable of producing new gene recombinations and variations as the original species. It would seem to follow that a homogamic complex is not specially limited in its evolutionary potentialities. The original species may or may not become extinct, but the group as a whole is not predestined to decline. It may, on the contrary, continue to evolve and give rise to new evolutionary developments.

There is a second distinction between the homogamic complex and the other type of hybrid complex, which Stebbins has pointed out in a personal communication. In the agamic, polyploid, heterogamic and clonal complexes the derived types may be easily recognized by their cytological and genetic behavior. It is consequently possible to identify such complexes even after the extinction of the parental species. But the recognition of a homogamic complex as such depends upon the contemporaneous existence of both the original and the derived species. We can recognize the last relics of agamic, polyploid, heterogamic and clonal complexes which have existed for many ages and have undergone many migrations and evolutionary changes, but we can only recognize the more recent and actively evolving homogamic complexes which have not suffered widespread extinctions.

The identification of the original species in a homogamic complex is a highly uncertain procedure. How is one to decide that a supposedly original species is not really the descendant of hybrids between older species which have since disappeared from the scene? The original species of one homogamic complex could very well be the final products of preexisting homogamic complexes. Even the most intensive study of a homogamic complex can permit only minimal estimates of the importance of hybridization in the evolution of such a group.

Anderson believes that hybridization has played a major role in the evolution of most agricultural plants and weeds. (Anderson, 1952). The Leafy-stemmed Gilias represent a group devoid of agricultural uses or weedy tendencies which has apparently been hybridizing since ancient times. It is fair to inquire how widespread the pattern of hybridization has been in the evolution of the Angiosperms as a whole. According to Stebbins (1947), approximately half of the species of flowering plants are polyploids. If, as seems probable, most natural occurring polyploids are allopolyploids (Clausen, Keck and Hiesey, 1945; Stebbins, 1947), then at least half of the species of flowering plants are of hybrid origin. This again is only a minimal estimate of the extent of hybridization.

Perhaps the most realistic appraisal of the influence of hybridization in the evolution of the Angiosperms can be gained from a consideration of the confused taxonomic structure of this class. The

process of adaptive radiation has brought about fairly clear-cut divisions between the major phyletic lines in most animal groups, not excluding the almost infinitely numerous and varied class of insects. As a result zoologists have been able to devise a generally satisfactory and standardized system of classification of animals. Several generations of plant taxonomists have been unable to duplicate this achievement for the Angiosperms. A prominent plant taxonomist has recently stated that "None of [the existing classifications of higher plants] begins to answer the requirements of a truly phylogenetic system, and no system of the foreseeable future can attain such a goal" (Lawrence, 1952).

Though a primary divergence in the Angiosperms between the Monocotyledons and Dicotyledons was recognized several centuries ago, the intensive activity of the 19th and 20th centuries has not led to the recognition of major lines of evolution diverging within these two groups. Consequently there is no generally accepted classification of the Dicotyledons and Monotyledons into orders. There is apparently a reticulate, rather than a dichotomous pattern of relationships among the families and orders of flowering plants. This reticulate pattern seems to characterize the relationships of even the most primitive families of Angiosperms (Bailey and Nast, 1945; Money, Bailey and Swamy, 1950).

The reticulate taxonomic structure of the flowering plants can be explained by a modification of an hypothesis of Stebbins (1947), which holds that hybridization was of widespread occurrence during the early phases of angiosperm evolution, just as it has been in the more recent evolution of gilias, ceanothi, columbines, oaks, brambles, agricultural plants and weeds. This hypothesis is consistent with the known facts concerning the frequency of polyploidy in the Angiosperms. It accounts for the lack of clear discontinuities between the major phyletic lines by postulating the occurrence of interbreeding between those lines during the early stages of their divergence. It supposes that the Angiosperms have evolved, not along exclusively divergent lines, as seems to have been the case in most animal groups, but as a succession of hybrid complexes. The occurrence of sporadic hybridizations during the course of Angiosperm evolution may be the factor which has caused this group to grow up, not as a phylogenetic tree, but as a gigantic, snarled phylogenetic net.

Since their origin the Angiosperms have explored many new ways of life and have pursued long continued trends of specialization in both their vegetative and reproductive characters. This occurrence of an open system of evolution is probably consistent with the presence of a reticulate pattern of relationships between families and orders only if it is assumed that the Angiosperms have comprised a succession of homogamic complexes. This is because the homogamic complex is the only type of hybrid complex in which hybridization can continue to occur without resulting in the imposition of restrictions on the free recombination of genes in the derived species.

The complexity of the angiospermous phylogenetic net is no doubt increased by polyploidy, as suggested by Stebbins (1947, 1950). An unknown proportion of the families and genera may have arisen from tetraploids which have come to behave genetically like diploids. Most of the higher categories, however, have probably originated from diploid stocks. These ancestral stocks may have been hybridizing on the diploid (or diploidized) level since the earliest stages of Angiosperm evolution.

Summary

Groups of organisms in which hybridization has blurred the morphological discontinuities between the basic species are

collectively designated as hybrid complexes. Hybrid complexes may be subdivided according to the mode of reproduction of the derived forms into five main types, namely agamic, polyploid, heterogamic, clonal, and homogamic complexes. In homogamic complexes the hybrid derivatives are diploid, sexual populations with a normal chromosome cycle at meiosis, rather than apomicts, polyploids, permanent structural heterozygotes, or clones. There is consequently much less restriction on gene recombination in the derivatives of homogamic complexes than in the end products of agamic, polyploid, heterogamic or clonal complexes. As a result, homogamic complexes possess an open system of evolution, as opposed to the closed system of evolution which characterizes the other types of hybrid complex.

Evolution in a homogamic complex is illustrated by the Leafy-stemmed Gilias (Polemoniaceae), a cluster of related species of annual herbs which grow sympatrically in California. The species are sexual with a normal meiotic cycle and are predominantly diploid. They are well isolated from one another in nature by a variety of external and internal isolating mechanisms. Yet they seem to intergrade in a manner which makes them exceedingly difficult to classify. There is evidence that the taxonomic complexity of the group is due to occasional hybridization between populations which under most circumstances are well isolated. The Leafy-stemmed Gilias exemplify a homogamic complex, in which hybridization could continue indefinitely without reducing the ability of the group to make progressive evolutionary advances.

The Angiosperms as a whole have undergone many progressive evolutionary changes while developing as a phylogenetic net rather than as a conventional phylogenetic tree. In the light of independent evidence for a widespread occurrence of hybridization in the past history of the Angiosperms, this combination of a reticulate pattern of relationships with progressive trends of specialization suggests that the class may have evolved as a succession of homogamic complexes.

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CROSSING AND SEXUAL ISOLATION OF EGYPTIAN FORMS OF *MUSCA DOMESTICA* (DIPTERA, MUSCIDAE)[1]

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INTRODUCTION

House flies are an important factor in the public health of Egypt. In order to assess fully the best methods for control of flies in Egypt, the United States Naval Medical Research Unit No. 3 and other institutions have undertaken studies on insecticides and bionomics of these insects (Peffly, 1952). As soon as these studies were begun, it became apparent that there was little agreement among taxonomists concerning the various forms of *Musca domestica* in Egypt. Morphological characters tended to be confusing, and biological differences were poorly understood. It was therefore decided to attempt to clarify these matters on the basis of (1) field and laboratory biological studies, and (2) investigations of crossing and sexual isolation. In conjunction with the latter phase, a specialist in muscoid taxonomy made a brief field survey chiefly to advise on diagnostic morphological characters (Sabrosky, 1952).

Although the typical form, *Musca domestica* L., must be frequently introduced into Egypt via ships and planes, it has seldom or never been recognized there. The chief forms in Egypt are *vicina* Macq. and *cuthbertsoni* Patton. They were considered valid and distinct species by Van Emden (1939), but Sabrosky (1952) tentatively regarded them as subspecies of *domestica*. These forms would not be regarded as subspecies by Mayr (1942) because they occur in the same geographic area. Sabrosky believed flies from Egypt identified as *nebulo* Fab. to be merely occasional variant individuals.

Van Emden (1939) differentiated these forms from typical *domestica* and from each other chiefly by variations in length and strength of postsutural dorsocentral bristles (in both sexes) and by relation of frons width to width of the third antennal segment of males.

Van Emden separated the forms in this complex as follows:

1. All postsutural dorsocentral bristles strong 2

   The anterior 2–3 postsutural dorsocentral bristles weak, obviously shorter and weaker than the two nearest the scutellum; frons of male about as wide as the third antennal segment or narrower; abdomen bright orange, not or only slightly infuscate on the fourth segment, median vitta narrow on third segment and usually absent on fourth..... .....*Musca cuthbertsoni* Patton

2. Male frons [4] about three times as wide as third antennal segment; abdomen largely infuscate in female, at least third and fourth segments infuscate in male..... .....*Musca domestica* L.

   Male frons [4] less than three times as wide as third antennal segment; abdomen more or less bright orange, especially at sides of base, usually darkened on fourth segment and at least apex of third in male .....*M. vicina Macq.* (Including form *nebulo* Fab.)

[1] The opinions or assertions contained herein are the private ones of the writer and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

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[4] Van Emden demonstrated a gradual transition between *domestica*, *vicina* and *nebulo*.

Sabrosky (1952) suggested that, instead of the relation between frons width and third antennal segment, the relation between frons width and head width be used. In the studies described herein, this relationship and the bristle character were used.

In general, a male specimen having long and strong postsutural dorsocentral bristles and a frons width about 10 per cent of the head width would be identified as *vicina*. A specimen having the first two or three pairs of postsutural dorsocentrals short and frons width about 5 per cent of head width would be identified as *cuthbertsoni*. These percentages were determined from measurements of laboratory strains (tables 1 and 2). In *cuthbertsoni* the ratio of frons width to head width was 0.046 (range 0.019–0.093, S.D. 0.012), in *vicina* this ratio was 0.092 (range 0.042–0.120, S.D. 0.013).

Effect of Interbreeding of Differentiating Taxonomic Characters

Laboratory strains of *vicina* and *cuthbertsoni* were crossed to determine the effect of these crossings on characters used to differentiate the two forms. The *vicina* strain was obtained from an Egyptian village near Cairo and the *cuthbertsoni* strain from a suburban slaughter house. When these experiments were started, the *vicina* strain had been reared in the laboratory for over 70 generations and the *cuthbertsoni* strain for over 20 generations.

Flies newly emerged from the laboratory strains were segregated by sexes. Individuals were selected for long- and short-bristledness. To make the selections, flies were immobilized with carbon dioxide and observed through the medium power of a dissecting microscope. A suitable male and a suitable female were placed in a 3- by 9-inch cylindrical screen cage and provided with food (milk-soaked cellucotton).

The record of each pair included bristle measurements, date of pairing, dates of egg laying, and number and sex of emerging flies.

To determine the normal range in bristle ratios of each type, long-long pairings and short-short pairings were made. Long- and short-bristled flies were paired

Table 1. *Percentage distribution of the ratio of the first to the fourth postsutural dorsocentral bristle of laboratory strains of* Musca vicina *(long) and* M. cuthbertsoni *(short) and crosses between the two*

Ratio	Long × long	Short × short	Long × short (F_1)	Int.[5] × int. (F_2)	Int. × int. (F_2)	Int. × long	Int. × short
.101–.150	—	1	1	1	1	—	1
.151–.200	1	3	2	1	3	2	1
.201–.250	1	6	2	3	3	2	5
.251–.300	1	12	3	4	3	—	14
.301–.350	—	26	5	2	5	1	19
.351–.400	—	19	9	4	5	1	14
.401–.450	3	14	10	8	11	4	18
.451–.500	8	10	22	18	23	12	8
.501–.550	10	5	21	16	18	18	11
.551–.600	27	4	14	25	13	21	8
.601–.650	29	1	11	14	9	22	1
.651–.700	16	—	2	6	5	13	1
.701–.750	5	—	—	—	1	4	—
.751–.800	1	—	—	—	—	—	—
Number of flies	176	235	666	177	186	256	80
Mean	.591	.368	.480	.510	.477	.558	.394
S.D.	.084	.097	.107	.113	.126	.108	.112

[5] Int. = intermediate, progeny of crosses between longs and shorts.

TABLE 2. *Percentage distribution of the ratio of the frons to head width of males of laboratory strains of* Musca vicina (*long*) *and* M. cuthbertsoni (*short*) *and crosses between the two*

Ratio	Long × long	Short × short	Long × short (F_1)	Int.[6] × int. (F_2)	Int. × int. (F_3)	Int. × long	Int. × short
.011–.020	—	1	—	—	—	—	—
.021–.030	—	3	—	1	—	—	—
.031–.040	—	30	—	2	—	—	—
.041–.050	1	29	1	5	1	—	12
.051–.060	2	28	12	10	10	—	12
.061–.070	1	6	29	8	18	2	37
.071–.080	13	2	26	19	10	14	10
.081–.090	24	—	17	9	17	19	17
.091–.100	29	1	9	29	5	18	7
.101–.110	28	—	5	10	21	34	5
.111–.120	4	—	1	6	8	11	—
.121–.130	—	—	—	—	10	1	—
.131–.140	—	—	—	—	1	—	—
.141–.150	—	—	—	2	—	—	—
Number of flies	136	139	470	108	189	135	41
Mean	.092	.046	.075	.083	.089	.096	.070
S.D.	.013	.012	.014	.022	.022	.014	.016

[6] Int. = intermediate, progeny of crosses between longs and shorts.

as well as two generations of their progeny. Progeny of long-short crosses were also backcrossed.

Unless needed for further pairings, emerging flies were not fed and consequently died in about 1 day. Twenty-five of each sex were pinned and placed in a Schmidt box. Measurements were made of the first and fourth postsutural dorsocentral bristles, of frons width at its narrowest point, and of the head at its widest point. The ratio of the length of the first to fourth bristle of each specimen and of frons to head width of each male was calculated. Averages of these ratios and their percentage distribution were determined. Intervals of 0.05 and of 0.01 were used to distribute the bristle ratio and frons-head-width ratios, respectively.

The results of these calculations are shown in tables 1 and 2.

Although there was overlapping between the two forms, each had a distinct and separate distribution of ratios. Most of the progeny resulting from crosses between the two forms had bristle ratios intermediate between the two parent forms, but more had long bristles than short ones. In second- and third-generation progeny the distribution of these ratios was almost like that of the first generation. Progeny produced from backcrosses tended to fall into the type group to which the backcross had been made.

The results of these crosses on frons-head-width ratios of males were equally distinctly marked. It should be recalled that original crosses were made between flies selected for a given bristle character. Short-bristled flies produced narrow-frons specimens, long-bristled flies produced wider frons. Progeny of crosses between the two had intermediate frons-head-width ratios. Second and third generations had ratios distributed over the entire range, though most fell into the wider-frons, long-bristled group. Progeny of backcrosses to shorts tended to have narrow frons, those of backcrosses to longs had wider frons.

These studies provide information on what may be expected in field collections. If the two forms do interbreed in nature, as they readily did in these tests, most progeny would appear to be *vicina*.

Because the range of both the bristle

character and frons-head-width ratio of each group overlaps, the group to which "intermediate" specimens belong cannot readily be determined.

Sexual Isolation Tests

In the previous tests flies of the two kinds were confined together and were forced to mate. While these tests were in progress, it was noted that pairings of short-bristled flies were not so successful as those of long-bristled ones. More time was required to obtain the first viable eggs and a smaller percentage of pairings resulted in viable eggs. Dr. Dobzhansky, who was consulted on this problem, suggested that male-multiple-choice tests with flies two generations removed from the field would better determine the degree of sexual isolation, if any.

Dobzhansky (1950) pointed out that it was not sufficient evidence to report that two forms interbred and produced fertile offspring in the laboratory. Many insects, when "forced" in the laboratory, do just that. What really matters is not whether Mendelian populations do or do not exchange genes in nature, but, if they do, whether it is at a rate that destroys the adaptive equilibrium of the population concerned.

Colonization of field strains: Field collections were made at two locations, a Cairo dump and a suburban slaughter house. Both types of flies were present at both places, but *vicina* (long-bristle) was predominant at the dump and *cuthbertsoni* (short-bristle) at the slaughter house.

Females with the desired bristle character were isolated in 3- by 9-inch cylindrical screen cages. The food material, milk-soaked cellucotton, was examined daily for eggs or larvae. Eggs or larvae were counted and placed in CSMA media at the rate per egg or larva of 1.5 grams of the mixed media. Adults reared from them were selected for further propagation. Enough flies were used to provide an ample number of flies for tests with the subsequent generation.

At first progeny of each isolated wild female were kept separate. This procedure entailed a great amount of labor and space. It was observed that differences in mating behavior of the two strains were so constant that the additional work of keeping isolated strains from individual wild females was discontinued. Thereafter, groups of selected females from the two field sources were isolated. This procedure was less arduous and also guaranteed a constant supply of both types of flies for multiple choice and other tests.

Difference in mating propensity: In male multiple-choice tests it is important that only 50 per cent of the females be inseminated. Several series of tests were run to determine conditions under which 50 per cent insemination might be expected. Half as many males as females were used. Longs were confined with longs and shorts with shorts. With laboratory strains 50 per cent insemination occurred between 2 and 3 days.

With flies two generations from the field, the long-bristled type mated readily but the short-bristled type did not. Newly emerged flies were confined in cages for 2, 3, 4, and 5 days. No short females were inseminated in 3 days, but 86 per cent of the long females were. Only 19 per cent of the shorts were inseminated in 4 days and 17 per cent in 5 days.

In other tests the sexes of newly emerged flies were segregated and united, longs with longs and shorts with shorts, after 1, 2, 4, 5, or 6 days. On the following day the females were dissected. Under these conditions 50 per cent of the long-bristled flies were inseminated between the third and fourth days, but even when 7 days old only 25 per cent of the shorts were inseminated.

In the above tests flies taken at random from each strain were used. It has been our experience, however, that there is a considerable amount of overlapping of bristle ratios between the two strains. Therefore, the experiments were repeated

TABLE 3. *Mating propensities of long- and short-bristled flies. Five males and 10 females used in each test. Ten tests each 24 hours long*

Source	Age (days)	Bristle type	Percentage of females inseminated	
			Average	Range
F_2-field	4	Short	6	0–30
		Long	65	40–100
	8	Short	10	0–40
		Long	60	30–80
Laboratory	4	Short	80	50–100
		Long	87	60–100

with flies which were individually examined before use. Short-bristledness was considered to be 0.4 or less, *i.e.*, the length of first bristle no more than 0.4 that of last bristle. Flies were considered as long bristled when this ratio was 0.5 or more. Selected 3-day and 7-day old flies were placed in 600-ml. beakers, covered with stockinet, and were held for exactly 24 hours.

The results (table 3) show that long-bristled flies mated much more readily than short ones. Insemination among short-bristled females did not reach 50 per cent. Increased age to 7 days did not alter the mating propensity of either type. Laboratory strains of both types mated much more readily than flies only two generations from the field.

Male-multiple-choice tests: In male-multiple-choice tests, as described by Bateman (1949), equal numbers of two strains of females are enclosed in one cage with half as many males of one of these strains. In another cage males of the other strain are enclosed with both types of females. After a lapse of time sufficient to give 50-per cent insemination, the distribution of inseminated females between the strains is observed. The sum of the interstrain matings divided by the sum of the intrastrain matings is the sexual-isolation estimate (Merrell, 1950). When the estimate is 0, complete isolation exists; *i.e.*, no interstrain matings occur. If the estimate is 1, no isolation exists, *i.e.*, mating between both types occurs at random.

In each of the male-multiple-choice tests described in this paper, two cages were used. In one cage, 10 short- and 10 long-bristled females were confined with 10 long-bristled males. In the other cage, 10 females of each of the two strains were confined with 10 short-bristled males.

Male-multiple-choice tests with flies confined in 600-ml. beakers, although showing a high degree of sexual isolation between the two forms, were not completely satisfactory, largely because of lack of mating by short-bristled flies.

In similar tests using 1-cubic-foot screen cages, there was considerable more breeding of the short-bristled strain. The results (table 4) are of tests with flies that were 3 days old when the tests were begun and 7 days old when they were finished. The two forms are sexually isolated. The isolation was especially significant with selected second- and third-generation flies and with unselected third-generation flies. By the fourth generation, however, especially with unselected flies, so many more than half the females were inseminated that the tests were of no value in showing isolation. The reason for the isolation shown was the considerably greater amount of mating among long-bristled flies.

Tests with selected flies: A colony of short-bristled flies was maintained at the laboratory for more than 20 generations. In order to keep the colony short-bristled, it was important to select parents having short bristles. Otherwise the short-bristled character was suppressed by long bristledness. It is true that long-bristled flies are closely related genetically to the short-bristled flies in this strain, and it would seem that they should act similarly with regard to their mating propensity. Male-multiple-choice tests and mating-propensity tests were run with succeeding generations of both strains to determine whether selected and unselected flies reacted differently—that is, whether

TABLE 4. *Summary of male-multiple-choice tests of long- and short-bristled flies. Flies 3 to 7 days old during tests*

Generation from field	Type of male	Ratio of inseminated to total females		Per cent of total females inseminated	Merrell isolation estimate	Adjusted chi square
		Short	Long			
		Flies selected for bristle character				
Second	Short	17/84	8/92	14		
	Long	12/89	82/92	52	0.202	29.75**
Third	Short	29/91	41/96	37		
	Long	26/92	91/97	62	.558	6.88*
Fourth	Short	32/46	24/46	62		
	Long	24/48	39/45	68	.676	3.59
		Flies not selected for bristle character				
Second	Short	3/26	0/27	6		
	Long	9/26	22/30	55	.360	3.32
Third	Short	77/137	61/133	51		
	Long	56/136	120/127	67	.594	17.32**
Fourth	Short	24/30	23/29	80		
	Long	19/28	28/29	82	.807	.686
Fourth[7]	Short	59/110	45/109	47		
	Long	50/110	64/109	52	.772	3.107

[7] Flies 3–4 days old during tests.

long- and intermediate-bristled flies in the short-bristled strain mated just as their short-bristled sibs.

Table 4 shows that over 10 per cent more interstrain mating occurred among unselected flies than among selected ones. This resulted in a higher sexual isolation estimate. By the fourth generation so much mating occurred that the tests were not valid. That is, so many more than half the females were inseminated that the point at which sexual isolation could be demonstrated was exceeded. When 3- to 4-day tests were made with this generation, close to 50 per cent insemination occurred, but there was so much interstrain mating that the sexual isolation estimate was high.

In mating-propensity tests 5 males and 10 females were confined together, long females with long males and shorts with shorts. Flies were selected until the fourth generation; in later generations it was difficult to find enough short-bristled flies in the short colony. Until the third generation no marked differences were shown between the number of selected and unselected females that were inseminated. By the fourth generation, however, especially among short-bristled flies, a marked difference was shown. It appears then that there may be an association between bristle length and mating propensity in the short-bristled strain. Long-bristled flies from the short strain mated more readily than their short-bristled siblings.

Table 3 shows a difference in the number of inseminated females between long- and short-bristled field strains. The table also shows that, after many generations in the laboratory, the mating propensity of both strains increased until there was very little or no difference between them. It was noted that erroneous conclusions might be made if laboratory rather than field strains were used.

Mating-propensity tests were run with

TABLE 5. *Percentage of females inseminated by their own kind of males in succeeding generations in the laboratory*

Generation from field	Short-bristled females		Long-bristled females	
	Selected	Unselected	Selected	Unselected
F_2	6	0	65	53
F_3	0	0	65	78
F_4	6	37	55	73
F_5	—	34	—	63
F_6	—	45	—	64
F_7	—	67	—	88
Laboratory colony	80	—	87	—

flies from each generation from the second to the seventh. As shown in table 5, the percentage of inseminated females in the short strain was markedly higher in the fourth generation than in previous generations and continued at the higher rate in the next three generations. No marked change occurred among the long-bristled flies.

Loss of short-bristled character during continued rearing of field strain: After the laboratory strain of *cuthbertsoni* was started from short-bristled specimens, it was observed that many progeny had long bristles. It was found that in order to keep the colony short-bristled, all parents had to be selected for short-bristledness. During the summer and autumn, ample numbers of short-bristled females were collected from the field source of this strain, but when the weather became cooler, fewer and fewer short-bristled females were collected. The percentage of shorts present in collections from June to September was 70 per cent; it decreased to 50 per cent in October, to 15 per cent in November, to 7 per cent in December, and to 0 in January.

As previously described, wild females were selected for short bristles and their progeny were also selected for the same character. As a result, most second-generation flies were short-bristled. No further selection of parents was made in succeeding generations. The length of the first and fourth postsutural dorsocentral bristles of 50 males and 50 females in each generation from the first to the seventh was measured. The findings (table 6) show that higher percentages of long-bristled flies occur in successive generations of the original short-bristled strain, but that the distribution in the original long-bristled strain changed only slightly from generation to generation.

The distribution of frons to head width ratios of succeeding generations (not in-

TABLE 6. *Per cent distribution of ratios of the first to fourth postsutural dorsocentral bristles of field strains of long- and short-bristled flies with continuous laboratory rearing*

Ratio	Long strain						Short strain					
	F_2	F_3	F_4	F_5	F_6	F_7	F_2	F_3	F_4	F_5	F_6	F_7
.051–.100	1	—	—	—	—	—	—	—	—	—	—	—
.101–.150	1	—	—	—	—	—	2	—	—	—	—	—
.151–.200	—	—	—	—	—	—	3	—	—	—	—	—
.201–.250	—	—	—	—	—	—	6	—	—	—	1	—
.251–.300	—	—	—	—	—	—	19	—	1	2	—	—
.301–.350	1	—	—	—	—	—	23	7	3	2	1	—
.351–.400	—	—	—	—	—	—	23	17	3	2	3	—
.401–.450	1	—	—	2	—	1	15	14	12	9	12	4
.451–.500	2	8	1	1	3	2	6	27	13	14	13	8
.501–.550	9	9	5	9	4	10	2	12	13	13	9	21
.551–.600	24	31	30	23	14	31	1	14	20	25	23	21
.601–.650	27	29	39	30	38	22	—	5	15	16	18	23
.651–.700	23	19	21	28	26	24	—	4	17	13	13	18
.701–.750	9	4	3	7	14	9	—	—	3	3	6	5
.751–.800	2	—	—	—	1	—	—	—	—	1	1	—
.801–.850	—	—	1	—	—	1	—	—	—	1	—	—

TABLE 7. *Per cent distribution of ratios of frons to head width of males from field strains of long- and short-bristled flies with continuous laboratory rearing*

Ratio	Long strain					Short strain				
	F_2	F_4	F_5	F_6	F_7	F_2	F_4	F_5	F_6	F_7
.021–.030	—	—	—	—	—	2	—	—	—	—
.031–.040	—	—	—	—	—	19	—	—	—	—
.041–.050	—	—	—	—	4	28	6	2	12	10
.051–.060	—	—	—	—	—	35	24	5	—	—
.061–.070	3	—	—	2	20	9	12	21	16	18
.071–.080	13	2	—	22	—	3	24	26	20	20
.081–.090	27	4	2	8	32	3	12	21	4	14
.091–.100	35	20	20	24	14	1	10	12	36	28
.101–.110	15	28	22	26	12	—	8	10	8	6
.111–.120	7	30	22	10	14	—	4	3	4	4
.121–.130	1	16	24	4	2	—	—	—	—	—
.131–.140	—	—	10	4	—	—	—	—	—	—
.141–.150	1	—	—	—	2	—	—	—	—	—

cluding the third) is shown in table 7. In the short strain this character did not change so rapidly as the bristle character, but by the fourth and following generations a higher percentage of wider frons appeared.

INTERBREEDING OF *vicina* AND *domestica*

Reasons and methods: Because of the difficulty in differentiating *vicina* and *domestica,* these forms have been regarded by many authors as a single species with world-wide distribution. However, Patton (1936) and Van Emden (1939) differentiated between the two. They claim that *vicina* apparently occurs throughout the warmer parts of the Old World, including Africa, India, and the warmer parts of the Palearctic region. It also extends into the New World, at least in the West Indies. *M. domestica* occurs throughout the Temperate regions.

According to Patton, *domestica* has never become established in Africa. This assertion arouses interest because *domestica* is undoubtedly introduced almost daily from ships and planes. Furthermore, breeding requirements of the two appear to be similar. In order to determine the potentiality of *domestica* survival in Egypt, pupae from a laboratory colony were obtained from G. Saccá of the Istituto Superiore di Sanità in Rome.

It is almost impossible to distinguish females of the two forms, but males differ in frons width; *domestica* males have a wide frons and *vicina* a narrow one. This differentiating character, however, is subject to quantitative variation.

Several methods of measuring the relation between frons width and other parts of the head have been used. Patton (1933) used the relation between frons and the width of the eye. In *domestica* the frons is approximately half the width of the eye, but in *vicina* it is approximately one-third. Van Emden (1939) used the relation between the frons and the third antennal segment. In *domestica* the frons of the male at the narrowest point is about three times as wide as the third antennal segment; in *vicina* it is less than three times as wide. Sabrosky (correspondence) suggested that it would be preferable to compare the narrowest width of the frons to the widest head width. He pointed out that the antennae are often curled or shrunken and easily knocked off. Furthermore, unless high magnification is used, one would be comparing measures of only a few units, and errors would be greater. In crosses reported herein the ratio of frons to head width has been used.

Rearing of all flies in these tests was done in CSMA media composed of bran and alfalfa meal to which yeast, malt, and water were added. Egg batches laid by

TABLE 8. *Per cent distribution of ratios of frons to head width of males in laboratory strains of* Musca vicina (*Mv*), M. domestica (*Md*), *crosses between the two, the F_2 generation, and the backcrosses*

Ratio	Mv×Mv	Md×Md	Mv×Md	×F_2	Backcross to Mv	Backcross to Md
.041–.050	—	—	1	—	—	—
.051–.060	—	—	—	1	—	—
.061–.070	2	—	2	1	1	—
.071–.080	15	—	4	4	3	—
.081–.090	29	—	13	21	17	—
.091–.100	34	—	28	25	43	4
.101–.110	15	—	21	24	24	12
.111–.120	3	1	19	15	7	48
.121–.130	1	7	8	6	3	16
.131–.140	1	17	3	4	1	16
.141–.150	—	18	—	—	—	4
.151–.160	—	17	—	—	—	—
.161–.170	—	17	—	—	—	—
.171–.180	—	12	—	—	—	—
.181–.190	—	6	—	—	—	—
.191–.200	—	4	—	—	—	—
Number of flies	147	208	277	140	144	25
Mean	.091	.154	.101	.101	.097	.119
S.D.	.012	.019	.016	.015	.011	.011

various females were counted, and each egg was allowed 1.5 grams of mixed media in rearing containers. Flies not needed for further mating tests were allowed to die in the rearing jars, and then collected, pinned, and placed in an insect box for measurements.

Series of *vicina* and *domestica* specimens were measured to obtain the range of each type. Crosses of the two types of flies were made, the eggs were planted, and the progeny collected and measured. First-generation hybrids were also crossed. Backcrosses to *vicina* and *domestica* were also made.

Results: The distribution of the ratio of frons to head width of males in various crosses is shown in table 8. Typical

TABLE 9. *Per cent distribution of ratios of frons to head width of females in laboratory strains of* Musca vicina (*Mv*), M. domestica (*Md*), *crosses between the two, the F_2 generation, and backcrosses*

Ratio	Mv×Mv	Md×Md	Mv×Md	×F_2	Backcross to Mv	Backcross to Md
.261–.270	—	1	—	—	—	—
.271–.280	10	—	2	—	—	—
.281–.290	12	2	—	1	2	4
.291–.300	34	4	12	10	13	16
.301–.310	22	23	42	16	30	44
.311–.320	18	30	24	32	46	20
.321–.330	4	28	19	26	7	16
.331–.340	—	11	1	13	2	—
.341–.350	—	1	—	2	—	—
.351–.360	—	1	—	—	—	—
.361–.370	—	1	—	—	—	—
Number of flies	50	252	116	92	46	25
Mean	.299	.317	.310	.317	.310	.308
S.D.	.013	.013	.011	.013	.009	.010

vicina and *domestica* males fall into two distinct groups with very little overlapping. When the two forms were crossed, most of the progeny fell in almost the same group as typical *vicina*. In a backcross to typical *domestica*, a trend to wider frons (*domestica*) was shown.

The distribution of these ratios indicates that the narrow frons character of *vicina* is dominant over wide frons of *domestica*. If a pair of these two types were to mate in nature, as these strains did under experimental conditions, the progeny would likely appear to be *vicina*. Furthermore, when these F_1 flies mated in nature in Egypt, where only *vicina* occurs, chances are great that F_2 progeny would appear to be *vicina*, not *domestica*.

It appears that the reason *domestica* as such has not become established in Egypt is that it loses its identity when breeding with native flies—*i.e.*, the narrow frons of *vicina* is a character dominant over the wide frons of *domestica*.

Ratios of frons to head width of female progeny in these crosses were also calculated (table 9). Even though there is a slight tendency for *domestica* females to have a wider frons than *vicina* females, the difference is so small that this character is of no value in differentiating the two types of females.

Summary

Laboratory studies of crossing and sexual isolation between some forms in the *Musca domestica* complex were made in an attempt to clarify some of the existing confusion in this complex in Egypt. Characters used to differentiate *vicina* and *cuthbertsoni* were the ratio of first to fourth dorsocentral bristle in both sexes and the ratio of frons width to head width in males. Laboratory strains of these two forms readily interbred and produced fertile offspring. Ratios of differentiating characters of progeny fell between those of the two parent forms, but tended to be closer to *vicina* than to *cuthbertsoni*. The ratio in progeny from backcrosses fell into the range of the type to which the backcross had been made. Results indicated that *vicina* characteristics are dominant over those of *cuthbertsoni*.

Sexual-isolation tests were run between the two strains of second, third, and fourth generations from wild fertilized females. In male-multiple-choice tests these two strains were sexually isolated in the second and third generations; by the fourth generation, though isolation was still evident, it was much reduced. The degree of isolation was due largely to the greater amount of mating by *vicina* and to the greater attraction of *vicina* males for *vicina* females.

The mating propensity of laboratory strains of those two subspecies was about equal. Wild strains, only two or three generations removed from the field, did not mate so readily as laboratory strains. This lack of mating propensity was marked in *cuthbertsoni*. After several laboratory generations *cuthbertsoni* manifested a greater mating propensity. The mating propensity of *vicina* changed little during the first seven laboratory generations. Biological tests with these forms should utilize flies fresh from the field.

Vicina-like siblings in the *cuthbertsoni* strain mated more readily than did siblings that appeared to be *cuthbertsoni*.

Without constant selection of parents, the short-bristled character of *cuthbertsoni* was dominated by long-bristledness of *vicina*. This tendency also carried over into the frons-head width ratios. Thus it may not always be possible to identify field-collected specimens to the correct form.

Male *vicina* and *domestica* were differentiated on the basis of frons-width and head-width ratios. When laboratory strains of the two were crossed, ratios of the progeny were almost those of typical *vicina*. In other crosses (F_1 and backcrosses) the same tendency was shown. Characteristics of *vicina* are therefore considered dominant over those of *domestica*. This fact may largely ex-

plain why *domestica* has never become established in Egypt.

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GEOGRAPHY, ECOLOGY AND COEXISTENCE IN RELATION TO THE BIOLOGICAL DEFINITION OF THE SPECIES

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Introduction

Mayr (1942, p. 120) defines the species biologically as follows: "A species consists of a group of populations which replace each other geographically or ecologically and of which the neighboring ones intergrade or interbreed wherever they are in contact or which are potentially capable of doing so (with one or more of the populations) in those cases where contact is prevented by geographical or ecological barriers." ("Intergrading or interbreeding" refers of course to primary intergradation, genetically unrestricted (Mayr, 1942, p. 99), not to the production of a few secondary hybrids or highly localized hybrid zones.) He also makes the important distinction between those species that overlap with very restricted or no interbreeding, which can be recognised as good species biologically, and those with completely separate ranges, for which only indirect evidence of their status is available. "Two forms or species are *sympatric* if they occur together, that is if their areas of distribution overlap or coincide. Two forms (or species) are *allopatric,* if they do not occur together, that is if they exclude each other geographically."

These definitions raise two important questions.

(1) What is the meaning of 'geographical' and 'ecological'; and in what sense can some populations replace each other geographically and others ecologically?

(2) When can areas of distribution be said to overlap? If these can be answered, certain ambiguities and difficulties of application of the definitions to borderline cases may disappear.

Meaning of 'Geographical' and 'Ecological'

(1) When two species are endemic, one on each of two separate islands, their ranges are undoubtedly separate, whatever the nature of the islands and of the medium that surrounds them. If the islands are important to man (land in sea, oasis in desert), they will have names, and the description of the species' ranges is easily made by reference to these names, that is, geographically.

(2) When two species occupy the one woodland the other grassland (for example) in a region where these two types of vegetation intermix, so that there are islands of woodland in grassland and vice versa, it is obviously far easier to describe their distribution in terms of the vegetation, that is, ecologically. And although only one species could be found in any one locality, their geographical ranges would be said to overlap.

(3) But when such species occupy woodland and grassland in a region where these two formations occupy compact provinces with only part of their boundaries in common, their ranges could be characterized ecologically or geographically with equal ease.

It is clear from these three examples that ranges that can be characterized readily merely by reference to *spatial* distribution over the surface of the earth are called *geographical,* while those for which reference to the distribution of edaphic, climatic or biotic factors provides the most convenient description are called *ecological;* and further these two classes of range are not exclusive but overlap widely, perhaps almost completely, as Mayr (1942 p. 229, 1947) has emphasized.

Geographical and Ecological Replacements

From Mayr's definitions, the first of the three examples just given is undoubtedly of allopatric species, but (2) and (3) are not certainly so. Mayr and Vaurie (1948) give an instructive example. In Celebes there are two forms of drongo, one (*Dicrurus montanus*) related to others in the Northern Moluccas, and restricted to hill forests, the other (*D. hottentottus leucops*) related to forms in Ceram, Buru, and the Sula Isles, and restricted to lowlands (Vaurie, 1949). Mayr and Vaurie remark "Plotted on a large-scale map the ranges of *montanus* and *leucops* on Celebes almost exclude each other. However, this pseudogeographical representation is due to differences in the ecological requirements. *D. h. leucops* is a bird of the lowlands and of the more open country up to 1200 meters, only occasionally in the true forests, while *D. montanus* is a forest bird and prefers higher altitudes (500–1800 m.). However, since there are no signs of interbreeding in the forest at the lower altitudes where the two forms occasionally come in contact, they must be treated as separate species. . . ." Although the replacement is characterized as only pseudogeographical, *D. montanus* is in practice regarded as one member of the superspecies *D. hottentottus*, and by definition all the members of the superspecies are geographically replacing forms (Mayr, 1931; 1942, p. 169). This arrangement of these drongos has considerable taxonomic value, but it is seen that *D. montanus* is regarded as ecologically separated when its specific status is in question, and as only geographically separated when its taxonomic arrangement with *D. hottentottus* is under discussion.

It is therefore necessary to try to give a more precise meaning to the idea of replacement when used in connection with estimates of specific status, and to determine when exactly two species can be considered to occur together.

Sympatry and Allopatry

In the first place it is obvious, as Mayr (1942) has pointed out, that since crossability in nature is the test of specific status, only the breeding ranges need be taken into account, and these only in the breeding season. Immature and nonbreeding individuals do not give valid evidence for overlaps in which interbreeding could take place. It might be suggested that species can be regarded as truly sympatric over some part of their ranges if, although there is no interbreeding, the breeding ranges of the forms considered meet or overlap somewhere so that there is no spatial barrier separating individuals of the two forms that they cannot overcome.

But it is far from easy to decide what can be overcome. Mayr (1942) describes the remarkable localisation of the superspecies *Zosterops rendova* in the Central Solomon Islands, where extremely distinct species and subspecies are confined to islands separated by only a very few miles of sea. The powers of flight of these birds are ample to allow them to cross these straits in a few minutes. There is therefore no physical barrier, and it seems reasonable to say that it is the intrinsic habitat preference of the birds that restricts them to such a remarkable degree. In fact here one has good geographical separation because of an intrinsic barrier.

In consequence, it cannot be said that organisms prevented from interbreeding by intrinsic factors are truly sympatric, since in this example they are obviously allopatric. The case of the drongos is less certain. Here again habitat restriction is probably important, and it should be noted that the evidence for overlap is confined to one immature male of *montanus* from Paleleh (Vaurie, 1949), one mature male of *leucops* from Mt. Massarang at 3000 ft. (within the general altitudinal range of *montanus* although not actually collected with it), and specimens of *leucops* at 800 m. and *montanus* at 1000 m. from Rurukan recorded by

Stresemann (1940). Stresemann lays no special emphasis on overlap; in fact, he stresses the sharp ecological distinction between these two forms, while adding that they might occur together occasionally or by chance. However, since Celebes is not well known, yet already these indications of overlap have been found, it seems probable that the edges of the ranges touch at least occasionally. Habitat preference, although very important, is probably not the only isolating mechanism.

In the *Zosterops rendovae* superspecies, individuals from different species never normally meet in nature; in the drongos of Celebes it appears that they probably do come close enough together for attempts at pair-formation to take place, and this is the essential difference between these two examples. The important point is to decide whether individuals (or their products) ever come into such proximity in their breeding seasons that all of the processes normally leading up to fertilization could reasonably be considered as possible. Let the word *coexistence* be used to denote such a state of affairs. Then sympatric species are those in which some individuals of each coexist (in this restricted sense) without hybridizing, allopatric species are those in which none coexist. The members of the *Z. rendovae* superspecies are allopatric by this definition, the Celebes drongos are sympatric.

It is obvious that the word *replacement* has been used to cover two distinct cases of very different evolutionary significance, illustrated by the examples given at the beginning of this paper. Example (1) is of complete geographical and ecological replacement (if the two islands differ ecologically) and of complete allopatry. Example (3) is of geographical and ecological replacement but of sympatry because of the contiguity of ranges. Because of the equal ease of description of the ranges by ecological and geographical terms there has been some confusion about the nature of the replacement. Example (2) would unhesitatingly be called a case of sympatry, although strictly speaking there is complete replacement of one species by the other at any locality. Genetical sympatry is compatible with geographical replacement if the ranges of the forms considered are contiguous.

A Modified Biological Definition of the Species

From the definitions of coexistence, sympatry and allopatry just given it follows that if two populations are completely allopatric, and never meet in nature in such a way that interbreeding is possible, one never knows their relative status; paradoxically enough, a population may be known with certainty to behave as a good species (as defined by Mayr) towards all sorts of other organisms, whose specific, ordinal or even greater distinction from it have never been questioned, while its relations to a second, isolated population which although not identical is yet obviously its closest existing relative may be completely uncertain. As Mayr has shown, it is a matter of opinion whether some groups of geographically separated (allopatric) forms constitute a single polytypic species, a single superspecies, or even a supergenus. Mayr uses the criterion of actual intergrading or interbreeding or the potentiality of it when allopatric populations are in question. This potentiality cannot be tested in the laboratory or experimental garden since may species are known that will not breed in artificial conditions, and some that do not interbreed in nature but will do so in the laboratory (Mayr, 1942). Evidence obtained from breeding is therefore not conclusive, although it may be very valuable. A comparison of all possible characters is all that is available for determining the relative status of allopatric forms: it seems better to recognise this fact frankly. Indeed, there is much to be said in favour of restricting the term subspecies to those populations that do actually meet and intergrade in nature, and referring to all

taxonomically distinct and wholly allopatric but closely related populations by some neutral terms, not by species or subspecies. The term semispecies (Mayr, 1942, p. 165) is available for this purpose.

A modified biological definition of the species can now be given:

At any one locality, groups of individual organisms may be found to coexist (so that interbreeding is possible), which groups each behave as genetically intraconnected populations, but are genetically separate or nearly separate from each other. Each such group shall be called a species. When such groups in different localities are found to be interconnected by spatially intermediate populations all such groups are referred to the same species. Unconnected (allopatric) groups may also be referred to the same species if it is considered that their similarity warrants this step.

Two points in this definition require further comment: (a) 'Locality' has here the meaning of any continuous area of the earth's surface (land or sea). There is no need to prescribe its size, since whatever locality is taken as a starting-point, extension from it will allow coverage of the geographical area of the species. (b) If secondary hybridization is taking place between populations it becomes a matter of opinion where species-limits should be set. A very restricted region of secondary hybridization along the common boundary of two forms should not be taken as an excuse to consider them conspecific. Where a considerable part of the combined areas of the forms is occupied by secondary hybrids, conspecificity may or may not be recognised. Even an extensive occurrence of sterile hybrids does not indicate conspecificity, since there is no actual gene-flow between the populations.

Botanical Terminology

Valentine (1949) and Baker (1952) have recently discussed some relevant botanical terms. It is worth while considering how these accord with the species definition just given. The principal specific and subspecific categories recognised by them are *coenospecies, ecospecies* and *ecotype*. The coenospecies is taken to be a population (or group of populations) incapable of exchanging genes with any other population—that is, if any hybrids are produced, they are sterile. The relationship of this meaning to Turesson's original usage is discussed by Turrill (1946). The coenospecies is therefore certainly a category of specific or supra-specific status.

The nature of the ecospecies has been reviewed by Valentine (1949), and by Baker (1952) who reprints a number of definitions. The central idea in almost all of these is that the ecospecies is a population (or group of populations) which is freely fertile within itself but is prevented in some way from a free exhange of genes with other populations. Ecotypes are defined by Valentine (1949) as "groups forming genetically distinct components of ecospecies, adapted to special types of environment and capable of unlimited gene-exchange."

Baker (1952) refers to "the generally held concept of isolation between ecotypes as purely ecological while that between ecospecies is partly ecological and partly 'genetic.'" He points out that with Valentines definitions, which allows this limitation of gene exchange to manifest itself under either natural or artificial conditions, there is no absolute distinction between ecospecies and ecotypes, since "even ecotypes are prevented from the *free* exchange of genes in nature by their adaptation to separate environments." The situation would appear from these authors to be as follows:

(1) If two forms are distinct in nature, and cannot be made to produce fertile hybrids artificially, then they belong to different coenospecies.

(2) If two closely related, but ecologically distinct forms do produce fertile hybrids occasionally when they meet in nature but both there and under artificial

conditions the hybrids show a reduced viability, then these forms are separate ecospecies within the same coenospecies.

(3) If two closely related but ecologically distinct forms either totally intergrade in nature where they meet, or appear completely interfertile under artificial conditions, they are ecotypes within the same ecospecies.

In addition, Valentine (1949) has subdivided ecospecies into *'g-ecospecies'* which are "groups with the same chromosome number between which there are well-defined morphological, ecological and geographical differences, and which, under artificial or natural conditions are capable of only limited gene exchange," and *'a-ecospecies'* (of abrupt as against gradual origin) which are "groups differing in chromosome number between which there are well-defined ecological and geographical differences and which are capable of only limited gene-exchange." The g-ecospecies are those ecospecies that are formed typically by geographical isolation, while the a-ecospecies arise suddenly by polyploidy and are immediately sterile or nearly so towards the parental diploid form. Valentine is prepared to consider polyploids as ecospecies even if there are no morphological distinctions, provided that they are distinct either ecologically or geographically, or both, from the diploids; but if there is no distinction other than chromosome number, then even though they may be completely isolated genetically, he considers that they cannot be called ecospecies. For such forms he used the term cyto-type.

It seems that these definitions have been produced mainly in relation to two phenomena. First, many Linnean species of plants are found to be divisible into several or many forms which may hybridize to some extent, but in general are true-breeding and often more easily distinguished ecologically than morphologically, *i.e.*, ecospecies. Secondly, localised forms, sometimes clearly adapted to a local habitat, are found which are genetically determined and completely interfertile with more widespread neighbours under artificial conditions and apparently in nature, and which tended to recur whenever particular combinations of edaphic or climatic conditions recurred, *i.e.*, ecotypes. Hence the insistence on ecological factors and the sharp distinction between taxonomic species and ecological ones.

From the foregoing discussion, it is obvious that the coenospecies certainly corresponds to the species as defined biologically but could also correspond to a genus, the constituent species of which hybridized slightly but regularly. The eco*specis* also corresponds to the biological species, and since no two species have exactly the same ecology and range, the prefix *eco* is unnecessary, except that 'ecospecies' as an equivalent to 'biological species' is often used in contrast to 'taxonomic species.' As in the biological species, if hybridization is taking place, one may expect every intermediate between a highly localized and weakly developed hybrid zone and almost complete fusion of the two forms over the greater part of their combined range. It is then a matter of opinion as to where the taxonomic line shall be drawn to separate two distinct but extensively hybridizing species, and a single new species of hybrid origin.

The eco*type*, the product of genetic response to a particular habitat, may be recognised taxonomically or not according to the distribution of the particular habitat within the area of the species. If it occupies one compact province, then the ecotype is exactly the same as the geographical subspecies, which also is a geographically definable product of local conditions. Every geographical subspecies of animal which shows the operation of Gloger's rule, for example, is an ecotype, since it is different from other subspecies in correlation with living in a different climatic region.

If, however, the habitat is widely scattered through the area, then a geographical definition becomes impossible, and it is

likely that the same form may have arisen many times in response to particular conditions. It is suspected that in some animals the same character, which is of subspecific value, has arisen on several occasions (*e.g.*, iris-colour in drongos, Mayr and Vaurie, 1948). In the very polymorphic snail *Cepaea nemoralis* (L.), the colonies from very scattered and widely separated beechwoods, for example, are far more similar to each other than to downland and hedgerow colonies in the intervening areas. (Cain and Sheppard, 1950). One could very profitably refer to the beechwood, oakwood, or hedgerow ecotype of *C. nemoralis*. As already mentioned, it is impossible to determine the exact status of geographically isolated populations, which may be ecotypes (subspecies), ecospecies (species) or coenospecies (species).

As Mayr has pointed out, what matters in the evolutionary history of two forms is whether they do in fact exchange genes in nature, not what they can or cannot be made to do artificially (although, of course, the possibility of making artificial crosses may indicate the potentiality of hybridization occurring in nature if conditions change); this difference between crossability in nature and artificial fertility or sterility is of the utmost biological importance (Mayr, 1942, p. 119). It is now beginning to be more generally appreciated. Valentine (1949) has recognised the importance of crossability, and Baker (1952) comments on the difficulties that arise when too much emphasis is placed on the results of artificial pollination and other unnatural processes. One might well extend Baker's recognition that, if natural gene-exchange is considered, there is no absolute distinction between ecotypes and ecospecies, by adding that there is no absolute distinction between ecospecies and coenospecies either. There is a complete series from those forms that could cross perfectly but never meet through those that can be crossed artificially but do not interbreed in nature to those that cannot and do not interbreed. As slight decreases in viability of hybrids cannot be detected in the experimental garden but might be very important in nature, and as a *total* inability to exchange genes cannot in fact be proved, the limits of the coenospecies, ecospecies and ecotypes cannot be observed.

The cytotype has a very different status. From the evolutionary standpoint no greater biological distinction than complete intersterility between two forms can well be imagined. On Valentine's definitions, some intersterile forms are given specific rank (coenospecies), others (cytotypes) are not. Yet biologically, cytotypes should all (unless completely interfertile with the diploid parents) have the same rank as ecospecies, or coenospecies. Moreover, the cytotype, like the sibling species, is only an extreme in a continuous series of species showing every degree of morphological difference, and can be granted a separate status only very artificially.

It seems that the reason for the apparently low status assigned to the cytotype is that if its specific rank is granted, it must have a name, but it cannot be separated from the diploid by any morphological character. Sturtevant's contention that *Drosophila pseudoobscura* and *persimilis* must be called *D. pseudoobscura* rate A and B because they are inseparable on museum material has a similar basis. But there is no objection whatever to the museum taxonomist labelling his specimens merely "*D. pseudoobscura* agg.," and leaving it at that. It would indeed be surprising if those methods of preservation that happened to be available to the biologist before 1800 (say) also happened to be completely sufficient for delimiting all the species-groups in nature.

Valentine (1949) suggests that the botanical terminology might be used with advantage in zoology. Mayr's 'biological species' is in fact always an eco- or coenospecies, but the distinction between these two, as already mentioned is hardly necessary. The introduction of *cytotype*

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with its present meaning is undesirable. The term ecotype, however, may well be found useful, as suggested above for *Cepaea*.

Other Sorts of Species

The biological definition of a species cannot apply to any purely asexual forms. Further, as Simpson (1952) has shown, it cannot exhaust the meanings of 'species' as understood in palaeontology since it takes no account of the passage of time. One might add that it has nothing whatever to do with those 'species' which are merely individual variants that happen to be common at a particular horizon and, being useful to the stratigrapher, have therefore been forcibly segregated and named. Wilmott (1950) has proposed the useful term *palaeospecies* for species which are short lengths of lineages; for a monophyletic succession of such species the term *gens* (Vaughan, 1905) is available. It is highly desirable that monographers and reviewers should indicate clearly the status of their species. There are in fact four main practical meanings of species, derived from:

(a) A group of specimens which is considered sufficiently different morphologically from the most closely related forms known, to be given a specific name.

(b) A group of specimens for which there is good evidence that they represent one stage in a gens, and which may suitably be delimited specifically within that gens.

(c) A group of specimens for which there is good evidence that they conform to the biological definition of the species.

(d) A group of specimens for which there is good evidence that they represent a *totally* asexual population of any description.

The first of these meanings clearly covers principally those specimens that for lack of evidence cannot be assigned to any of the others; as such, it has enormous practical value. For these meanings respectively may be used the terms *morphospecies, palaeospecies, biospecies* and *agamospecies* (in a wider sense than Turesson (1929) proposed). That all these terms are of hybrid derivation has not the slightest importance in biology. *Superspecies* and *subspecies* are derivatives of the *biospecies*. (The use of *superspecies* for the same concepts as *gens*, recently proposed by Sylvester-Bradley (1951) obscures two totally different concepts under the same name.) A careful use of these terms would enormously facilitate the use of any monograph.

Summary

The ideas of 'geographical' and 'ecological' as applied to the distribution of species are ill-defined and overlapping.

A revision of Mayr's biological definition of the species is proposed, which avoids the use of these terms.

This definition and its derivatives are compared with some specific and subspecific concepts used in botany.

The necessity for a clear recognition of the different meanings of species, by the use of distinguishing prefixes, is emphasized.

Acknowledgment

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NOTES AND COMMENTS

THE USE OF THE BINOMIAL EXPANSION FOR A CLASSROOM DEMONSTRATION OF DRIFT IN SMALL POPULATIONS

VERL L. HOUSE

Several authors (Dubinin and Romaschoff, 1932; Moody, 1947; Bonnier, 1947) have described models for classroom demonstration of the effect of "drift" in small populations. Of the three authors cited, Moody and Bonnier present procedures that best simulate conditions of bisexual reproduction. The methods of these two writers differ with respect to the manner of selection of mates and the manner of chance determination of offspring from matings, but both methods involve the determination of zygotic frequencies in succeeding generations of small populations. Since the attention in a classroom discussion of the role of drift in small populations is more apt to be focused on changes in gene frequency rather than on changes in the frequencies of particular zygotic types (*e.g.*, the decrease in heterozygosis), a model utilizing sampling from a gametic pool and bypassing the diploid stage would be useful. Such a model may be based on the assumption of a random union of gametes from a parental population producing the N diploid individuals of the subsequent generation.

A population of N individuals represents a sample of 2N gametes from the gametic population of the preceding generation. If only two allelic types, *A* and *a*, are considered, a random sample of 2N gametes will have a probability distribution in the succeeding generation given by the expression $[(1\text{-}q)A + qa]^{2N}$, where q is the frequency of the *a* allele, (1-q) that of the *A* allele, and N is the population size (Wright, 1931). We may take as our starting condition a group of similar, completely isolated populations (*e.g.*, island populations), each with the gene frequencies $p = q = 0.5$, and assume an absence of the systematic pressures of selection and mutation. Under these conditions the expansion of $(0.5A + .5a)^{2N}$ gives the percentage of populations possessing the gene frequencies O*a*, 1/2N *a*, 2/2N *a* . . . , 2N–1/2N *a*, 1 *a* in the first generation following random mating within each of the isolated populations. The fate of those islands which by chance obtain a particular gene frequency in the first generation will be given for the second and subsequent generations by the expansion of the binomial for p and q determined by the results of the previous sampling. Following the initial spread of frequencies from the $p = q$ starting condition, a continuation of the above process will lead to an equilibrium condition where the gene frequencies on 1/2N of the islands will reach fixation or loss in each generation (Wright, 1931).

The conditions specified above may be duplicated for classroom use by the employment of any device which permits the realization of the binomial probabilities for different values of p and q. The island model may be simulated by letting each student represent one of the original populations, and having him perform the sampling sequence until fixation or loss results. Each student therefore starts with a population where $p = q = 0.5$ and draws a sample of 2N gametes. He repeats this process for each generation, drawing the sample from the population of gametes specified by his draw of the previous generation, and continues this procedure until his sample contains either all *A* or all *a* alleles.

Sampling containers may be constructed out of a variety of materials. The *A* and *a* alleles may be represented by wooden beads of two colors and placed in the containers in the proportions of p and q of the gametic population desired. The sample of 2N gametes may be drawn from the population at random by shaking the container vigorously and reading the content of the sample at a sampling window or tube of such size as just to make visible 2N beads. This type of sampling makes possible a rapid succession of generations by avoiding handling of the beads by the student. The data to be described below were obtained by the use of containers constructed out of 7″ × 4.5″ rolled-oat cartons, each carton furnishing three such containers. The carton was cut into three lengths and the open ends closed up by means of bond paper and masking tape, leaving cellophane sampling windows of the desired size (fig. 1, A). More durable containers may be prepared by cementing together plastic cups, purchasable at any variety store. Transparent plastic tubes of various lengths may then be mounted on the container for the purpose of receiving the sample of beads following shaking (fig. 1, B). With $N = 2$ three containers are needed containing *A* and *a* in the ratios 3:1, 2:2, and 1:3. For $N = 4$ seven containers are necessary, containing *A* and *a* in the following proportions: 7:1, 6:2, 5:3, 4:4, 3:5, 2:6, 1:7. The speed with which data may be secured for the case $N = 2$ and the smaller number of containers necessary will usually dictate the selection of this case for demonstration, except where the results with different N values are to be compared.

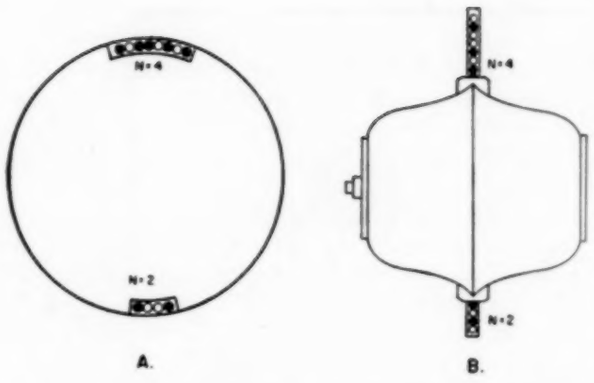

FIG. 1. Two types of sampling containers. A. Top view of card board container showing sampling windows for the cases $N = 2$ and $N = 4$. B. Side view of plastic container showing sampling tubes for the cases $N = 2$ and $N = 4$.

For the sake of clarity the actual procedure for the case $N = 2$ will be described. A student shakes his container containing equal numbers of the two types of beads (50 red and 50 green) and reads at the sampling window the genic content of his sample. After recording the content of this first sample he proceeds to a second container containing green and red beads in the ratio given by the sample previously drawn. For example, if the first draw shows 3 green beads to one red, the student next samples from a container containing green and red beads in the ratio of 3:1 (75 green: 25 red). This container is shaken and the colors of the four beads appearing at the window are again recorded. If the sample consists of either all red or all green beads the student terminates his run. Should the sample show a 1:1, 3:1, or 1:3 ratio, the student picks out the corresponding container and continues the sampling process until fixation or loss results. This process being repeated by a group of students furnishes data for each generation relative to the distribution of populations among the frequencies $4A:0a$, $3A:1a$, $2A:2a$, $1A:3a$, $0A:4a$. The plotting of these successive distributions by the student or instructor will furnish a graphic demonstration of the effects of small population size on gene frequency which may serve as a focal point for a discussion of the interaction of selection, mutation, and migration pressures with drift.

Figure 2 presents the results of 200 series for the case $N = 2$, the distribution of populations among the five possible gene frequencies

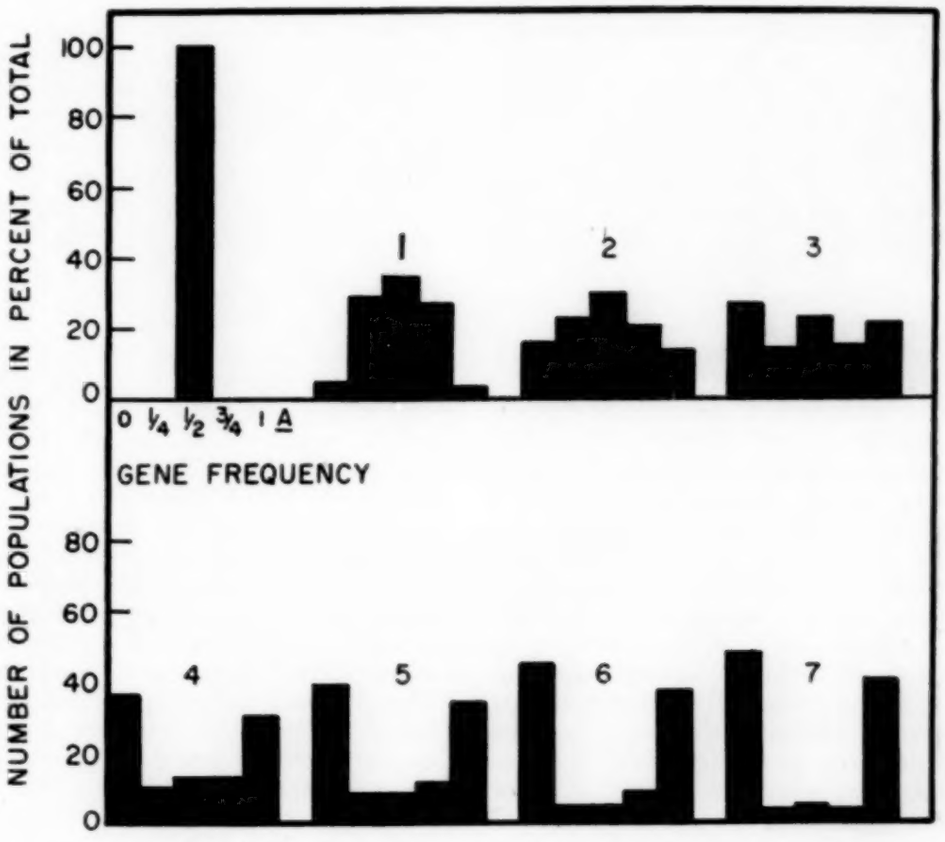

FIG. 2. Distribution of 200 initially identical populations according to gene frequency in successive generations of random sampling of gametes for the case $N = 2$.

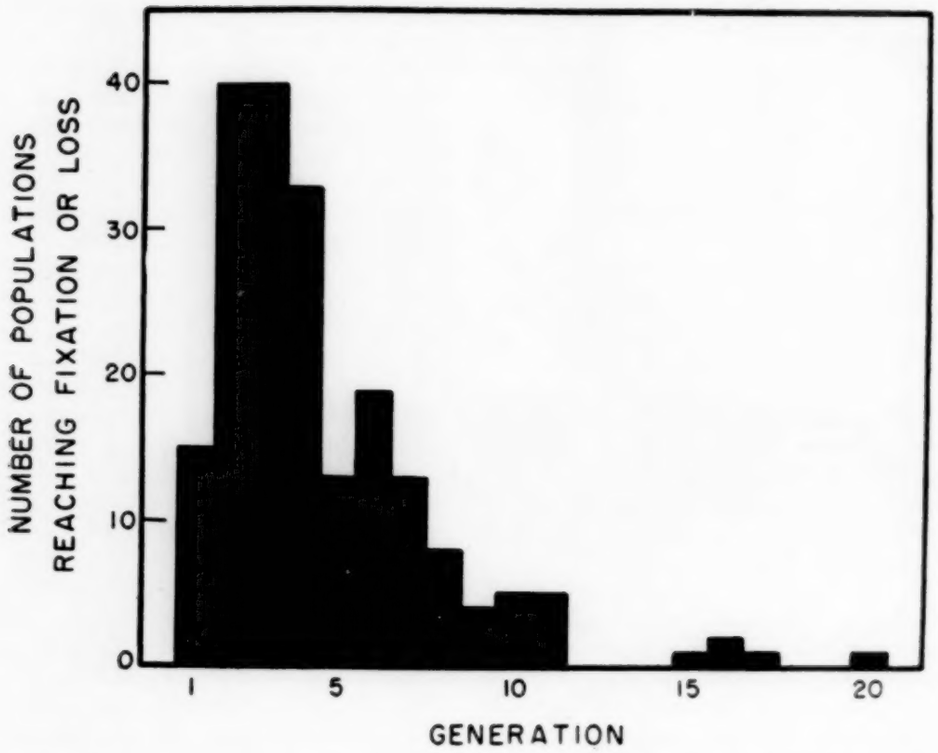

FIG. 3. Distribution of populations according to generation in which fixation or loss occurs.

being plotted for successive generations through the 7th generation. By the latter generation, chance has effected the fixation or loss of gene frequencies on most of the islands, only a relatively few possessing intermediate values of p and q. If desired it is possible to determine from the data the rate of fixation and loss occurring per generation. The present series gives an average figure of 28% fixation or loss per generation for the first seven generations, the initial spread of frequencies excluded. This value may be compared with the figure 1/2N (25%) mentioned earlier.

The results from such a run may be utilized in other ways which may be useful to beginning students in genetics. The data from the first sampling of the populations $p = q = 0.5$ may be plotted to demonstrate the binomial expansion $(0.5 + 0.5)^4$ (fig. 2, *1*); or the sampling from the populations $p = 0.75$, $q = 0.25$ may be summarized to demonstrate the binomial expansion $(0.75 + 0.25)^4$. A second form of the data which is useful in demonstrating to the student the progress of fixation and loss and the great variability in results among the different populations is that obtained by plotting the number of islands reaching fixation or loss in each generation (fig. 3). Figure 3 also demonstrates the ease with which this type of data may be accumulated in the classroom. Ninty per cent of the students have terminated their run by the eighth generation and all by the twentieth generation. Since it takes approximately five seconds to draw and record a sample by the described method (one generation), such data as the above can be accumulated in a few minutes. This leaves considerable time for plotting the data on the board by the instructor and for discussion, without making the exercise unduly long.

The interplay of "drift" with the systematic pressures of selection, mutation, and migration may also be demonstrated by the binomial model described above. In the case of genic selection we may assume that the alleles *A* and *a* are reproduced in the ratio 1:(1-s) per generation, where s is the selection coefficient (Wright, 1931). This systematic pressure tends to fix the *A* allele at a frequency of 1.0 and is opposed by any increase in q due to sampling. Starting with a population with the frequencies p*A* and q*a*, the frequencies for *A* and *a* in the generation following selection would be (1-q)/1-sq and (1-s)q/1-sq respectively, where 1-sq represents the residual population of genes following the selective elimination of *a*. We may incorporate the action of selection into our model in the following manner. The three containers having the alleles *A* and *a* in the ratios 3:1, 2:2, and 1:3 are replaced by three in which the ratios have been altered in accordance with genic selection of a given intensity. If $s = 1/4N = 1/8$ (for $N = 2$), the 1:1 container is replaced by one

having *A* and *a* in the ratio 8:7. The 3:1 and 1:3 containers would be replaced by populations of beads in the ratios 24:7 and 8:21 respectively. To demonstrate the simultaneous action of drift and selection each student would start with the 1:1 container and then proceed to those that have been altered by selection. For example, if the initial draw from the 1:1 population gives $3A:1a$, the next sample is drawn not from the 3:1 container but from the 24:7 container an so on. For each sample drawn the bias in the direction of the favored allele will be counteracted by the tendency to drift which results from the small sample of four gametes. The results of the combined drift-selection run may then be plotted as before. However, it will usually be sufficient to plot the distribution of gene frequencies at a given generation toward the end of the run rather than for each generation, as this will furnish an adequate demonstration of the effect of selection on the chance distribution of gene frequencies.

The effect of irreversible, recurrent mutation at the *A* locus may also be introduced into the model. The mutation rate u of $a \rightarrow A$ represents the fraction of *a* alleles that each generation transforms into *A* alleles. The frequency of *a* in the generation following the action of mutation will therefore be given by $q_1 = q(1\text{-}u)$. For the case $u = 1/4N$ the three containers $3A:1a$, $2A:2a$, and $1A:3a$ would be replaced by three having the following ratios: 25:7, 9:7, and 11:21.

It is the latter mutation model that has been utilized for the graphs shown in figure 4. Here are plotted the distributions obtaining in the eighth generation for the cases $u = 0$, 1/4N, 1/3N, and 1/2N. Where $u = 1/4N$ the same general type of U-shaped curve is obtained as when $u = 0$. With $u = 1/3N$ the systematic pressure begins to have effect with a skewing of the distribution in the direction of fixation of the *A* allele. When $u = 1/2N$, the effect of chance has been largely neutralized, most of the populations having become fixed for the allele *A* favored by the mutation pressure.

The degree to which the results from the type of series presented here are analyzed will depend to a large extent on the background of the students for whom the exercise is being prepared. Therefore different ways of handling the data obtained will no doubt suggest themselves to the reader. The sampling device described above may also be used for a variety of other purposes. Two such uses might be in connection with the introduction of the student to the concept of Chi Square and to the problem of genetic ratios in small families. A third use would be for the purpose of demonstrating the Poisson distribution. This series is given by the formula

$$e^{-\bar{x}}\left(1, \bar{x}, \frac{\bar{x}^2}{2!}, \frac{\bar{x}^3}{3!}, \cdots, \frac{\bar{x}^j}{j!}, \cdots\right)$$

where $\bar{x}$ is the mean. The Poisson distribution can be approximated by the binomial expansion, $(p+q)^n$, when p or q is relatively small in comparison with n. Figure 5 gives the distribution obtained for a series of 200 samples from a container containing red and green beads in the ratio 180:20 ($p = 1/10$), where the sample was read at a sampling window holding 20 beads ($n = 20$). The number of green beads appearing in each sample drawn was recorded and the frequency of samples containing 0, 1, 2, . . . 7 green beads has been plotted in figure 5. One characteristic of the Poisson distribution is that its variance is equal to its mean, *i.e.*, $\bar{x} = \sigma^2$. For the data of Figure 5, $\bar{x} = 1.93$ and $\sigma^2 = 1.48$. A comparison by means of χ^2 of the series of figure 5 with that expected from the Poisson formula gives a probability value < 0.10 but > 0.05 of obtaining a fit as poor or poorer on a basis of chance. The agreement is sufficiently close to warrant this use of the binomial in demonstrat-

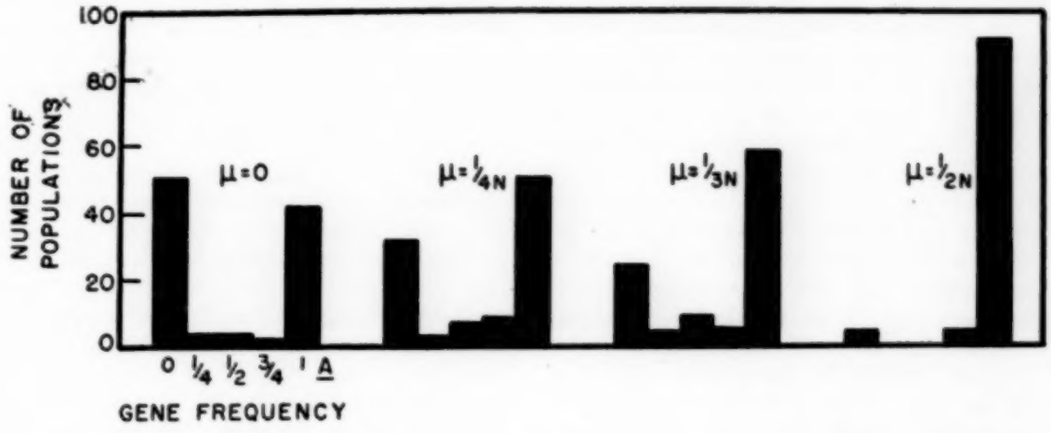

FIG. 4. The effect of different intensities of mutation pressure for the case $N = 2$.

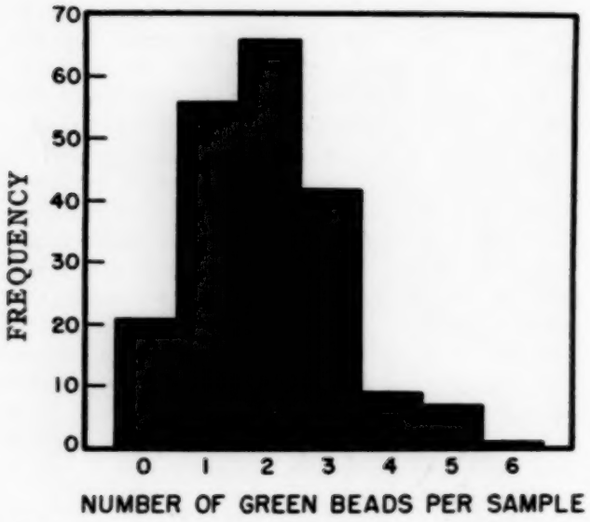

FIG. 5. The approximation of the Poisson distribution by means of the binomial expansion.

ing the characteristics of the Poisson distribution.

The versatility of the binomial expansion as a teaching aid, the rapidity with which results may be obtained with the described apparatus, and the ease with which the sampling containers can be constructed may recommend the binomial model to the teacher as a simple and efficient method for the laboratory treatment of elementary problems of population genetics and statistics.

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ON THE CHROMOSOMES OF THE HOG-NOSED SNAKE, *HETERODON PLATYRHINOS* LINNAEUS

RICHARD A. EDGREN

The publication of the second edition of Makino's atlas of animal chromosomes (Makino, 1951) points out our lack of complete knowledge regarding the karyology of even the most common animals. This general dearth of information prompts publication of the following notes on the chromosomes of the common hog-nosed snake, *Heterodon platyrhinos* Linnaeus, made incidentally during a systematic study of the genus (Edgren, 1952a, b). The original plan was to include an extensive comparative analyis of karyotypes for the species within the genus, but lack of suitable material forced abandonment of the project. The present notes were obtained from hematoxylin and eosin stained sections of a single testis from a specimen from Hillsboro County, Florida (RAE 1327).

Studies of metaphase figures indicated the presence of 6 V-shaped chromosomes, 8 to 10 I-shaped chromosomes and about 6 minute dot-like chromosomes. Thus the haploid number for the species is about 20–22. Measurements which were attempted on the 6 V-shaped chromosomes agreed fairly well from one series of measurements to the next (all taken in metaphase I). These 6 chromosomes fell into four size classes, one large chromosome, *ca* 5.5 μ; one somewhat smaller, *ca* 4.8 μ; two intermediates, *ca* 4.2 μ; and two small, *ca* 3–3.2 μ.

The V-shaped chromosomes appeared to be metacentric, although some measurements and observations indicated slight differences in the length of the arms. The I-shaped chromosomes all appeared to be telocentric as no short-arms could be resolved. The dot-like micro-chromosomes appeared spherical, although their extremely minute sizes precluded accurate observations.

G. D. Searle and Company,
Chicago 80, Illinois

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EXPLOSIVE EVOLUTION[1]

EDWIN H. COLBERT

In 1949 a symposium entitled "Distribution of Evolutionary Explosions in Geologic Time" was sponsored jointly by the Paleontological Society, the Society of Vertebrate Paleontology and The Geological Society of America. The symposium has recently been published in the Journal of Paleontology, and the papers comprising it are available in a separate binding.

Lloyd G. Henbest outlines the purpose of the symposium, which is "to examine the foundation in paleontology for the principle that diastrophism is the ultimate basis for dividing geologic history." Does the evidence of the fossils indicate rhythms in the waxing and waning of animal phyla and lesser categories that can be correlated with supposedly world-wide crustal movements occurring periodically throughout geologic history?

Henbest indicates the many difficulties and problems that arise in attempting an analysis such as this. Thus the reconstruction of the history of past life is subjected to many limiting factors, of which a few may be mentioned. For instance, the study of fossils has been greatly affected by the location of fossil deposits in relation to centers of education and research. It has been affected also by factors of preservation and geographic distribution, as well as by the gaps in our knowledge of the geologic record. In addition, allowances must be made for the accuracy or lack of accuracy in stratigraphic correlations, for the factor of homotaxis and for our interpretation of past ecological conditions. Differential rates of evolution are also a problem. Again, the dissimilarities in the geologic record in different continental areas must be taken into account and interpreted. When these and other qualifying factors are studied, it is apparent in the opinion of the author that theories of earth rhythm, so strongly advocated by some modern geologists, have exceeded the paleontological evidence.

After an exposition by J. P. Marble of the lead method for the measurement of geologic time, and some of the problems involved in this method, the following authors in the symposium proceed to examine "evolutionary explosions" and their relationship to the events of historical geology.

G. Arthur Cooper, Alwyn Williams, Raymond C. Moore and Norman D. Newell give interpretations of evidence as based upon fossil invertebrates, while Charles L. Camp and George Gaylord Simpson consider the evidence of the fossil vertebrates. Cooper and Williams base their conclusions upon a study of the brachipods, while Moore is concerned with the crinoids. Newell takes a more inclusive subject, that of periodicity in all of the invertebrates through time. Of the two vertebrate paleontologists, Camp presents a discussion of the relationships between geological boundaries and faunal changes, while Simpson deals with the large subject of periodicity in vertebrate evolution.

All of these authors agree that among the animals with which they are familiar there were periods of rapid evolutionary development, when species, genera, families and higher categories proliferated through comparatively short spans of geologic time. Whether such developments should be called "explosions" is a moot point, but their relative effect was in most cases a very sudden increase, so that use of the term "explosive evolution" has connotations understood by most workers in the field.

The conclusion reached separately by the different authors participating in the symposium is that in a general way evolutionary explosions or eruptions occurred throughout geological time, and can not be closely correlated with the so-called diastrophic revolutions that have been supposed to control the periodicity of events through earth history. However the participants have varying opinions as to the details of evolutionary explosions through time. Thus Moore and Newell would link evolutionary outbursts among many invertebrates with the spread of shallow water seas over wide regions, and would explain this connection of events as the result of broad evolutionary developments taking place when new ecological environments were made available to marine animals. However Moore calls attention to the generally local nature of such outbursts in both time and space, a fact that is particularly stressed by Cooper and Williams on the basis of their brachiopod studies. "The history of the brachiopods is characterized by evolutionary bursts and . . . these are distributed in time serially and without very clear relation to the geologic periods. The bursts also show considerable provinciality. . . ." Newell sees periods of great evolu-

[1] Distribution of Evolutionary Explosions in Geologic Time. A symposium with articles by Lloyd G. Henbest, J. P. Marble, G. Arthur Cooper, Alwyn Williams, Raymond C. Moore, Charles L. Camp, George Gaylord Simpson and Norman D. Newell. Journal of Paleontology, 1952, **26**: 298–394. Copies of the symposium, bound separately, may be purchased from Kenneth E. Caster, Secretary, The Paleontological Society, University of Cincinnati, Cincinnati 21, Ohio, at $2.00 each or at $1.75 each in lots of five or more.

tionary activity or outbursts alternating with periods of low evolutionary activity or stagnation, and he feels that these sequences are not at random. Thus, "In a large proportion of the major groups, times of low evolutionary activity tend to coincide. This indicates a common external cause, namely, the relatively complete occupation of available and accessible habitats." Such times of low evolutionary activity among the marine invertebrates were followed by widespread extinction, after which there were great evolutionary expansions as animals became adapted to new environments. The perods of expansion were the Cambrian and the Ordovician, the Mississippian, and finally the Triassic and Jurassic.

Moore indicates that the greatest expansions among marine invertebrates often occurred within the middle of geologic periods, when expanding seas reached their greatest extents. The vertebrates, on the other hand, show virtually no correlations with such periodic events, except to the degree that new continental connections allowed land-living animals to expand from one continental area into another at a relatively rapid rate. One sees here the differences that appear in the study of land faunas as compared with marine faunas.

Camp points out the great dissimilarity between the northern and southern hemispheres during the time when reptiles were evolving as the dominant terrestrial animals. The evidence shows that certain diastrophic events that are supposed to mark the division between great geologic eras in one region are hardly if at all apparent in the evolutionary record of animals in another region. Simpson, in considering the entire story of vertebrate evolution, concludes that while there have been periods of explosive evolution among different vertebrate groups, these can in no way be connected with periods of diastrophism. Rather he envisages evolutionary cycles among different vertebrate groups, marked first by the appearance of new adaptive types, followed some 25 to 50 million years later by peaks in the evolutionary development of these types. For the various groups of vertebrates the first appearances and the peaks of adaptation occur at different times in the history of the earth, and together they form a continuing history from the Ordovician period to the present.

One might summarize these papers as follows. Among the brachiopods there were parallel and successive evolutionary explosions throughout geologic history, in almost every period from the Ordovician to the present. Among the crinoids there were likewise successive expansions, with peaks in the Silurian, Mississippian and the Jurassic. Among invertebrates as a whole one can see successive evolutionary outbursts, with three great expansions or explosions taking place in the Cambro-Ordovician, in the Mississippian and in the Triassic-Jurassic respectively. Among the vertebrates as a whole there were likewise three crucial stages that were marked by great expansions or explosions of evolutionary development. The first was in the Devonian, when all of the major groups of fishes became established and when the first vertebrates first ventured out on the land. The second was in the Permian and Triassic, when there were decided changes involving extinctions, replacements and evolutionary expansions among the bony fishes and the reptiles. The third was in the late Cretaceous and the Tertiary, when there was a great decline among the reptiles and a tremendous evolutionary expansion among the replacing mammals.

From this it can be seen that the periods of evolutionary expansion among various groups of animals do not coincide. Consequently it must be assumed upon the basis of present evidence, that although the evolution of life was closely interrelated with the development of environments, it was not controlled by diastrophic movements. Therefore it must be said that the idea of accelerated evolution correlated with periods of diastrophic disturbance has little foundation in the evidence at hand.

HETEROSIS AND EVOLUTION [1]

G. L. Stebbins, Jr.

The thirty chapters which record in book form the papers presented at the Iowa State College conference on Heterosis, held during the summer of 1951, are chiefly of interest to geneticists and plant breeders. Many of them, however, discuss principles which are of basic significance for an understanding of evolution in terms of population dynamics, and some present new information about the evolution of certain cultivated plants, particularly maize.

The introductory chapter by Conway Zirkle traces the evolution of the concepts of crossbreeding and inbreeding, beginning with the Egyptian rulers and the Greek deities. After

[1] Heterosis, John W. Gowen, Editor. 552 pp. Iowa State College Press, Ames, Iowa. 1952.

tracing the complex relationships of Zeus, Hebe, and their associates, he remarks, "With immortals, backcrossing offered no real problems." Zirkle concludes from his study that all of the factual background for understanding heterosis was known before Mendel's work was accepted, but recognizes that heterosis as a genetic concept and a basis for plant breeding practices was developed later through the investigations of Shull, East, and Jones. In the second chapter of the book, Shull describes in detail his classic experiments and tells how they led him to formulate the concept of heterosis. The later development of this concept and its application to plant breeding is recounted by H. K. Hayes in the third chapter.

The remaining 27 chapters, each one by a different author, are on a great variety of subjects, some of which have little or nothing to do with heterosis. In this reviewer's opinion, the greatest weakness of the book is that these chapters are arranged in a completely haphazard fashion, with no regard to either the author or subject matter. This is particularly unfortunate since many of them deal with the same topics and contain very similar material, such as those by Jones, Dobzhansky, Crow, and Hull on the genetic basis of heterosis.

The student of evolution will be most interested in two sets of articles; first those dealing with theories concerning the nature and genetic basis of heterosis, and second those concerned with the evolution of cultivated maize. In regard to the genetic basis of heterosis, two theories were proposed, according to Zirkle, by pre-Mendelian breeders in a more or less general form, and these are still with us today, having almost equal acceptance. One of them, designated by Crow as the *dominance* or *dominance of linked genes* hypothesis, was formulated in its present form by D. F. Jones, who in the present volume restates his belief that this is the only hypothesis for which there is clear specific evidence. This states that heterosis arises because a hybrid between two strains having good combining ability possesses a maximum number of favorable dominant alleles. Dobzhansky, in a short chapter on the nature and origin of heterosis, designates this type of heterosis as mutational heterosis. Its widespread existence has long been recognized by geneticists, and one of the principal questions raised by authors of chapters in the present volume is whether or not some forms of heterosis may have other genetic causes. The chief genetic basis other than that of favorable dominant alleles is the phenomenon designated by Crow, Hull, and other authors of the present volume as overdominance, or the greater vigor and ability to survive of a heterozygote for two particular alleles as compared to homozygotes for either of the alleles concerned. In his article, Hull presents evidence from the experience of corn breeders and from mathematical analyses of their data which favors his concept of overdominance. LeRoy Powers, on the other hand, concludes from his data on tomato hybrids that overdominance does not contribute to their hybrid vigor. Buzzati-Traverso, in a discussion of several experiments on heterosis in populations of *Drosophila*, describes a gene for light colored eyes which in population cage experiments proved to have a higher selective value than its normal allele, but the heterozygote for this pair of alleles was superior to both homozygotes. This is regarded by Crow as one of the most convincing cases of overdominance. In his thorough discussion of this phenomenon, Crow points out that it need not be of general occurrence to be important in determining hybrid vigor. He concludes that favorable dominant alleles contribute much to hybrid vigor and may explain completely some examples, but that overdominance or single gene heterosis cannot be overlooked as a contributing factor, particularly in examples in which the hybrids "greatly exceed in fitness the equilibrium populations from which their parents were derived." This conclusion is well borne out by the concensus of evidence presented in the papers of this volume.

The role of hybridization in the evolution of maize is discussed in articles by Paul C. Mangelsdorf, by E. J. Welhausen, and by Edgar Anderson and W. L. Brown. Mangelsdorf shows how introgression of teosinte germ plasm into maize has affected the characters of maize varieties ever since early prehistoric times. He suggests from the results of his experiments that maize varieties "which have absorbed teosinte germ plasm in the past are now buffered against the effects of teosinte genes," *i.e.*, they show greater dominance in F_1 hybrids with teosinte than do maize varieties which have never been exposed to hybridization with teosinte in their ancestry. This probably results from artificial selection for productive maize types in introgressive populations.

Wellhausen shows that the most productive races of maize now being grown in the high plateaus of central Mexico are derived from a series of hybridizations between races adapted to different conditions, some of which have introgression with teosinte in their ancestry. These more recent races have a high yield only in certain areas recently opened to cultivation; they produce poorly or fail altogether in the regions occupied by their parental races. In these Mexican races of maize, therefore, hybridization has not produced any types which supersede their ancestors except under new conditions to which these ancestors are not adapted. Their superiority in yield is due partly to the fact that they are adapted to a

region with a good soil and a favorable climate. Some of their less productive ancestors appear to have possessed what some investigators have called bottleneck genes. These depress or inhibit growth, but are selected under certain conditions because they enable the race containing them to mature seed in an unfavorable environment. Wellhausen's work shows very well that improvement of maize through hybridization and selection, whatever be the method employed, is successful mainly insofar as it produces types well adapted to the environment in which the crop is grown.

Anderson and Brown present detailed evidence showing that Corn Belt maize arose through hybridization between two very different series of ancestral varieties. One of these, the Northern Flints, was cultivated by the aborigines of eastern North America, and the other, the Southern Dents, is largely of Mexican origin. By crossing a Northern Flint with a Southern Dent they have resynthesized a typical Corn Belt maize. They then conclude that the heterosis which is being used by commercial corn breeders in the United States is acquired, at least in part, "by mingling the germ plasms of the Northern Flints and the Southern Dents." Using this information, they have successfully selected inbreds for combining ability by scoring them, according to Anderson's hybrid index method, for resemblance to Northern Flints or Southern Dents. This is a fine instance of how genetic and evolutionary theories may be put to practical use.

EVOLUTION IN DROSOPHILA[1]

THEODOSIUS DOBZHANSKY

For more than a decade, the group of investigators at the University of Texas led by J. T. Patterson and W. S. Stone has held a world leadership in studies on the evolutionary biology of Drosophila. The results obtained by the group have been reported mostly not in the biological periodicals but in eight volumes of "The University of Texas Publications," the first of which appeared in 1938 and the most recent one in 1952. To those engaged in research on Drosophila the appearance of each of these volumes has been an important event. The principal contributions of the "Texas group" are now summarized and discussed in relation to those of other workers in an elegant book by Patterson and Stone. It goes without saying that every student of evolution will find in this treatise a treasure house of facts and a source of inspiration.

There is nothing final or definitive about this book. Perhaps its most remarkable quality is precisely that it conveys to the reader the exhilaration which comes from scientific discovery, together with realization that yet greater findings are in store for those who would look for them. From the beginning to the end the book describes research in progress and often research merely begun.

It takes two chapters and more than 100 pages to outline the salient features of the systematics and the geographic distribution of Drosophila. Only 30 years ago some 230 species of Drosophila were known; the number known to the authors is 613. The increase (due to no small degree to the work of the Texas Group and primarily of Patterson and Wheeler) assuredly does not exhaust the species diversity even in the Nearctic region which is relatively the best explored one (141 species). This reviewer would venture a guess that the 200 species recorded in the Neotropical region amount to only one half of the actual diversity. The Ethiopian region, with 33 species, is now the least well-known one. It is no underestimation of the importance of the work of the Texas Group to say that the present classification of the subgenera and of the species groups will probably be changed considerably as better knowledge is secured.

More than 200 pages are devoted to analysis of evolution of the chromosomal apparatus, the virilis species group being considered in most detail. The chromosomes are known to some extent in 215 species. Among them 86 species have 6 pairs, 52, 51, and 25 species have 5, 4, and 3 pairs respectively, 7 pairs being present in a single species. A chromosome set consisting of 5 pairs of acrocentric and 1 pair of microchromosomes is accordingly considered the phylogenetically primitive one. Multiplication of the centromeres has taken place in the phylogeny much less often than their loss. By far the most important source of change in the chromosome structure are paracentric inversions (*i.e.*, not including the centromeres). Nevertheless, pericentric inversions (including the centromeres), and probably some translocations, have also occurred. The view that five long and one short chromosome strands are the

[1] Patterson, J. T., and Stone, W. S., 1952. Evolution in the Genus Drosophila. 610 pp.; 74 figs.; 109 tables. Macmillan Company, New York. $8.50.

six "elements" which preserve their integrity throughout evolution is invalidated.

Analysis of reproductive isolating mechanisms which make Drosophila species genetically closed systems takes also between 150 and 200 pages, the virilis species group, for the study of which Patterson and Stone have been largely responsible, being again considered in most detail. Only some 20 years ago the genus Drosophila used to be considered unfavorable for studies on isolating mechanisms, since no borderline cases between race and species were known, and since only two species were known to produce hybrids which were absolutely sterile. The authors list 101 hybrids between species of Drosophila, more than three-quarters of which have been discovered in the Texas laboratory. It should be realized that this spectacular increase came about largely as a result of the substitution of the modern biological species concept for the old-fashioned morphological one. The genus Drosophila happens to be rich in groups of sibling species which are morphologically very nearly similar. Nevertheless, sibling species often show clear physiological and ecological differences, and, more important still, are reproductively isolated from each other. It is among siblings species that some of the most favorable situations for studies on nature and genesis of isolating mechanisms present themselves. Some of the most beautiful experiments described in the book under review concern sibling species. This provides an object lesson to those who still insist that a Drosophila must be dried on an insect pin before its species is identified. If we want to understand what species are and how they are formed it is useful to recognize them wherever they exist. And if we have to talk and write about these species it is useful to give them names, as the authors have quite properly done.

In a book concerned with problems which are being actively investigated by many workers controversial views are almost certain to be found. Conflict of ideas is a healthy sign in science: when evolutionists will agree about everything it will be time for them to switch to studying something else. Considerations of space permit only brief mention of some views expressed in the book which this reviewer is inclined to question. The authors doubt that natural selection would build complete reproductive isolation between species, since preservation of some gene exchange seems most advantageous for progressive evolution (p. 549). Now, so broad a generalization is hardly warranted at present; on the contrary, it seems that limited gene exchange between species is adaptively favorable in some groups, particularly among plants, while the isolating mechanisms are more rigid in others. This variety of conditions may well depend on whether the organisms are wholly or only partially relying on sexual reproduction for their perpetuation, whether the numbers of zygotes produced in each generation are great or small, whether the genotypes are internally balanced systems or the effects of most genes are additive, and whether the environment is changeable or relatively static. The authors consider the gene exchange "possible" in 97 out of 156 species hybrid recorded in Drosophila (p. 423); the reviewer feels that gene exchange under natural conditions is completely excluded between most of these species pairs. Compared to many genera, especially among plants, Drosophila shows remarkably complete isolation between most species. The interpretation of heterosis (pp. 296–302) is likely to be rendered obsolete by rapidly accumulating evidence, which emphasizes the importance of coadapted gene complexes in the production of hybrid vigor. Geneticists, at least those working with higher organisms, will probably be led toward realization of the importance of the internal balance of gene systems in Mendelian populations, and away from the idea that such populations are essentially uniform and homozygous for most loci, except for scattered mutants.

The book of Patterson and Stone joins a group of several masterful presentations of the modern evolutionary thought published within the last ten years or so. Its contribution is likely however to prove more enduring than those of most other books of this group.

SOCIETY FOR THE STUDY OF EVOLUTION

Seventh Annual Meeting, Ithaca

Report of the Secretary for 1952

Minutes of the Council Meeting: held on September 8, 1952 at 4:00 p.m. in Room 284, Martha Van Rensselaer Hall, Cornell University, Ithaca, New York.

In Attendance:

President: E. B. Babcock
Secretary: T. Just
Council Members: S. Wright, E. Mayr

The Secretary presented the results of the election. The group then discussed several changes and additions to the Constitution and By-Laws and instructed the Secretary to formulate them and subsequently mail them to the Council for approval. The recommended changes and additions concern the automatic membership of retiring presidents on the Council for periods of three years and the responsibility of presidents to organize symposia for the annual meetings. In the absence of the Editor, Dr. E. H. Colbert, his report was presented by Dr. E. Mayr. The Editor's request for $7500 for Volume VII of Evolution (1953) was approved together with his list of Associate Editors (Class of 1955). The group recommended that botanists be included in the Class of 1956.

In the absence of the Treasurer, Mr. C. M. Bogert, his interim report was presented by Dr. Mayr and approved by the Council.

Dr. Mayr reported on the results of the membership drive. A circular letter was mailed to members of the Society of Systematic Zoology and forty new members were added.

The Secretary was instructed to secure bids concerning the cost of printing the mailing list in Evolution or having it published separately by varitype method, and to report his findings to the Council.

The Secretary reported that the Society had been invited to meet with the A.I.B.S. and the A.A.A.S. and asked to determine its meeting places for the next two or three years. The Council felt that long range commitments of this kind should not be made and voted to meet in 1953 at Madison, Wisconsin, under the auspices of the A.I.B.S.

Business Meeting: September 9, 1952.

The Business Meeting was held at 9:00 a.m. in the Amphitheatre of Martha Van Rensselaer Hall. The Secretary announced the results of the election and the decision of the Council to hold the meeting in 1952 at Madison, Wisconsin. The officers are:

President: Alfred S. Romer, Harvard University
Vice Presidents: A. Buzatti-Traverso, Universita di Pavia, Italy
William M. Hiesey, Carnegie Institution of Washington
Carl Hubbs, Scripps Institution of Oceanography
Secretary: F. Harlan Lewis, University of California
Editor: Everett C. Olson, University of Chicago
Councilmen: I. W. Bailey, Harvard University
T. M. Sonneborn, Indiana University

Respectfully submitted,
Theodor Just, *Secretary*

Correction

In the article by J. Ives Townsend entitled "Genetics of Marginal Populations of *Drosophila willistoni*," Volume VI, Number 4, an error occurred on page 435. The following correction is to be made in the second paragraph of column 2, lines 8 and 9:

Delete lines reading: "homozygotes with male homozygotes from the same F_3 culture; some other tests"

Substitute: "homozygous, caused sterility of one or both sexes. Some of their fertility tests"